



Air Pollution and Climate Change Effects on Grassland Ecosystems

Mark Lee

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy and the Diploma of Imperial College London

Department of Ecology and Evolution

Imperial College London

Silwood Park Campus

Ascot

January 2012

Abstract

Grassland ecosystems extend across a substantial area of the world's surface, providing many valuable ecosystem services including carbon sequestration, biodiversity preservation and food provision. Global and local environmental changes are anticipated in the future, including shifts in climatic conditions and changes to the composition of the atmosphere. This thesis adds to our understanding of how grassland communities respond to air pollution and climate change using two key methodologies. Firstly, a suite of environmental variables were measured in calcareous grassland ecosystems along transects adjacent to roads. This allowed quantification of plant compositional changes and identification of the likely drivers of plant compositional changes nearer roadsides. Evidence of road-derived changes to air quality, soil biogeochemistry and hydrology at roadsides are presented. The key messages being that road proximity was associated with increased abundances of nitrophilic species and also of species not typical of calcareous habitats. Secondly, a mesotrophic grassland ecosystem was exposed to a factorial combination of end-of-century rainfall regimes (+ 15 % winter rainfall and – 30 % summer rainfall) based on IPCC 4th Assessment projections and nitrogen enrichment. Plant productivity and species composition were resistant to nitrogen enrichment throughout the three year study. Above-ground plant biomass declined in rainfall manipulated plots by the third year, with evidence of increasing forb abundance and declining grass abundance. These data can assist projections of grassland responses to environmental change in the future and inform management decisions aimed at preventing decline in natural grasslands and declines in the ecosystem services that grasslands currently provide.

Acknowledgements

This study was funded by the Big Lottery Fund through the Open Air Laboratories project (www.OPALexploreNature.org).

I am eternally grateful to Sally Power, Linda Davies, Pete Manning, Emma Green, Ellen Fry, Charlie Marsh, Georgina Southon, Nathan Callaghan and Nigel Bell for comments on this thesis and for advice throughout my time at Imperial College.

Thanks to Kevin Clemitshaw and David Ames for use of their laboratory for NO₂ diffusion tube analyses and for making my visits to Royal Holloway so enjoyable.

Thanks also to Beniot Disch for assistance with soil heavy metal analysis, Constance Tragett and Tom Veitch for assistance with arthropod species identification and to Nadia Schwimmler for assistance with the collection of root data.

This work would not have been possible without the approval and assistance of Linda Smith, Robert Lloyd, Tim Speller, Robert Silverwood, Steve Tillman, Charlotte Williams, Leo Jennings, Angela Watson, Rachel O'Hara and Michael Allen who allowed work to be carried out and monitoring equipment to be left over several years at their beautiful calcareous grassland sites. Thanks also to the technicians of Silwood Park who helped construct the rainout shelters.

Special thanks go out to all my family and friends for their support and keeping me sane over the years and for the many good times, and thanks to the amazing Catherine Walker - for everything.

Finally, this work is dedicated to Jim Hudson who made me choose the right path so many times. Without Jim I would not have made it here and this thesis would not exist.

Declaration

I herewith certify that all material in this dissertation which is not my own work has been properly acknowledged.

Signed.....Mark Lee.....

Date.....23 April 2012.....

Contents

1	Abstract	2
2	Acknowledgements	3
3	Declaration	4
4	Tables and figures	8
5	1. Introduction	12
6	1.1. Global environmental change	13
7	1.1.1. Climate change and grassland ecosystems	14
8	1.1.2. Nutrient cycling and grassland ecosystems	17
9	1.1.3. Interactions between nutrient cycling and climate	19
10	1.2. Environmental change: the contribution of roads and road vehicles	21
11	1.2.1. Air quality alongside roads	22
12	1.2.2. Soil biogeochemistry changes at the roadside	27
13	1.2.3. Response of grassland plants to pollutant exposure	29
14	1.2.4. Responses of grassland plant to HM enrichment	31
15	1.2.5. <i>In-situ</i> effects on vegetation	32
16	1.3. Calcareous grasslands as roadside study systems	34
17	1.4. Aims and objectives	36
18	1.4.1. Road effects on grassland ecosystems	36
19	1.4.2. Climate and N effects on grassland ecosystems	37
20	1.5. Thesis outline	38
21	2. Effects of roads on adjacent plant community composition and ecosystem	
22	function: an example from three calcareous ecosystems	41
23	2.1. Abstract	41
24	2.2. Introduction	42
25	2.3. Material and methods	45
26	2.3.1. Study sites	45
27	2.3.2. Sampling technique	46
28	2.3.3. Air quality	46
29	2.3.4. Heavy metals in the soil	47
30	2.3.5. Soil pH and moisture	47
31	2.3.6. Extractable nitrogen and mineralisation	48

32	2.3.7. Vegetation surveying	48
33	2.3.8. Statistics	49
34	2.4. Results	51
35	2.4.1. Atmospheric NO ₂ and NH ₃ concentrations	51
36	2.4.2. Heavy metals in the soil	53
37	2.4.3. Soil moisture, pH and extractable N	54
38	2.4.4. Plant composition and productivity	56
39	2.5. Discussion.....	60
40	2.5.1. The influence of roads on air quality	60
41	2.5.2. Road induced changes to the soil physical and chemical environment ...	61
42	2.5.3. Road induced changes to adjacent vegetation	63
43	2.6. Conclusions	65
44	3. Direct and indirect effects of roads and road vehicles on the plant community	
45	composition of calcareous grasslands.....	66
46	3.1. Abstract.....	66
47	3.2. Introduction	67
48	3.3. Materials and methods.....	70
49	3.3.1. Sites.....	70
50	3.3.2. Soil HM concentrations.....	72
51	3.3.3. Soil pH, salinity and moisture content.....	72
52	3.3.4. Modelling NO ₂ concentrations and N deposition.....	73
53	3.3.5. Model testing	74
54	3.3.6. Vegetation surveying	74
55	3.3.7. Statistical Analysis	75
56	3.4. Results	77
57	3.4.1. Modelled gaseous N and N deposition	77
58	3.4.2. Road derived changes to the soil physical and chemical environment	78
59	3.4.3. Ellenberg scores, species richness and functional group shifts	81
60	3.4.4. Responses of individual plant species	85
61	3.5. Discussion.....	88
62	3.5.1. Modelling gradients of pollution deposition away from roadsides	88
63	3.5.2. Edaphic contribution to roadside plant compositional change	89
64	3.5.3. Identifying the key drivers of roadside plant compositional changes	90
65	3.6. Conclusions	93

66	4. Plant and arthropod community sensitivity to climate change and nitrogen	
67	enrichment in a successional grassland ecosystem.....	94
68	4.1. Abstract.....	94
69	4.2. Introduction	95
70	4.3. Materials and methods.....	98
71	4.3.1. Site and treatments.....	98
72	4.3.2. Soil moisture, extractable nitrogen and mineralisation.....	101
73	4.3.3. Plant composition and biomass	102
74	4.3.4. Arthropod community.....	103
75	4.3.5. Statistics	103
76	4.4. Results	104
77	4.4.1. Soil biogeochemistry.....	104
78	4.4.2. Species richness, plant composition and biomass production	107
79	4.4.3. Arthropod community.....	110
80	4.5. Discussion.....	112
81	4.6. Conclusions	118
82	5. General discussion.....	119
83	5.1. Overview of key findings	119
84	5.2. Roads.....	120
85	5.2.1. Edaphic and atmospheric conditions at roadsides.....	120
86	5.2.2. Modelling nitrogen dioxide concentrations away from roadsides	123
87	5.2.3. Practical application – Management of calcareous grasslands.....	124
88	5.3. Grasslands and global change.....	127
89	5.3.1. Opportunities and challenges in understanding grassland resistance and	
90	resilience to climate change	127
91	5.4. Comparing mesotrophic and calcareous grassland responses to	
92	environmental change.....	129
93	5.5. Conclusion	132
94	6. References.....	133
95	Appendix	160

Tables and figures

Table 1.1: Summary of plant composition and function responses to declines in annual rainfall (by manipulation of rainfall regime) in grassland ecosystems. Mean annual rainfall (MAR), mean average temperature (MAT) are also given. 16

Table 1.2: Dry deposition velocities (V_d) of exhaust gases from the air to plant surfaces. V_d for HMs is represented by Pb and is for indicative purposes. 23

Table 1.3: Summary of studies measuring air quality at different distances perpendicular to roadsides including the traffic densities of the roads that the study considered, the distance from the road that background concentrations were reached and the maximum concentrations (c) of NO_2 and NH_3 measured at roadsides. Na refers to unmeasured variables. 25

Table 1.4: Summary of air quality targets for the protection of vegetation and ecosystems in the UK (adapted from DEFRA, 2007). AOT40 = sum of the excess hourly concentrations greater than $80 \mu\text{g m}^{-3}$ (40 ppb). 26

Table 1.5: Summary of transect studies in ecosystems perpendicular to roadsides identifying which environmental variables were measured (x) and correlated (*) with plant species composition or foliar chemistry in each study system. Effect zone represents the stated zone of roadside influence. Soil parameters: extractable (ext) N, heavy metal concentrations (HM), total soil carbon (C) and total nitrogen content (N). 33

Table 1.6: Summary of N critical loads in grassland ecosystems (Bobbink & Hattelingh, 2011). Critical loads relate to the maximum deposition of N that an ecosystem is able to sustain without harmful effects. 35

Table 2.1: A summary of the sites used in this study including approximate road completion date and the distance (m) from the edge of the road to the first location marker. Annual vehicle density relates to mean annual vehicle numbers obtained from the nearest traffic count point over the ten years ending December 2008 (DfT, 2011). Traffic density is in million vehicles. 45

Table 2.2: A summary of regression analyses showing relationships between the stated variable and distance from the roadside. + denotes that the variable increases with increasing distance from the roadside, – denotes a decline with increasing distance from the roadside and ns denotes non-significance at $p > 0.05$. Linear, logarithmic (log) and quadratic (quad) denote the shape of the relationship ($n = 29$). 57

Table 2.3: A summary of the minimal adequate model from the multiple regression analyses for each site. Terms in initial multiple regression model: measured mean NO_2 , modelled mean NH_3 , and: moisture, pH, extractable soil N (ext soil N) and their interactions ($n = 29$). Optimal initial models were selected based on the model with the highest r^2 values. Relationships given are linear. Logarithmic and quadratic relationships were also tested but explained less of the variation in the data. 59

Table 3.1: Details of study sites including; annual traffic volume (T, million vehicles), management type (grazing, G and/or cutting, C), distance from the edge of the site to the road, elevation of the first location marker above (+) or below (-) the road, slope away from the road (incline, $\uparrow$, decline, $\downarrow$ or none, $\rightarrow$), location and rainfall. Conservation designations are: National Nature Reserve (NNR), Site of Special Scientific Interest (SSSI) and Area of Outstanding Natural Beauty (AONB). Mean temperatures range from 9 °C to 10 °C with annual rainfall given (Met Office, 2011). Grazing rates are given as the number of sheep per hectare (cattle per hectare at NC). 71

Table 3.2: Mean soil concentrations of Zn, Pb and Cu at each site measured nearest and farthest from the road. Physical barriers relate to a row of trees between the road and the grasslands or that the site is raised above the height of the grassland. Bold typeface highlights highest and lowest values. Means are derived from 3 replicates. 79

Table 3.3: Summary of the minimal adequate model (MaM) for the LME (cover) and GLM (species richness) analyses across all sites. Terms in initial model: moisture (M), modelled dry NO_2 deposition (NO_2 dep), modelled dry NH_3 deposition, total N deposition (dry + wet) and their interactions (:). pH was not significant in any model and was omitted. Log denotes that logarithmic transformation was required to obtain the model which best fit the data. The remaining terms are untransformed. 84

Table 3.4: LME model output across all study sites to show the plant species which increased/decreased in abundance nearer to roadsides. Species with Ellenberg scores for pH, moisture (M) and nitrogen (N) above 5 are indicated with an “x”. Species which are categorised as typical calcareous (C) and typical edge species (E) are also indicated with an “X”. 87

Table 4.1: Summary of mean summer and winter rainfall received in the four years ending December 2009 prior to commencement of the study and throughout the study in ambient and rainfall manipulated plots. 99

Table 4.2: ANOVA analyses summarising plant responses to nitrogen (N) or rainfall manipulation (Rainfall) when compared with controls (No N and ambient climate). Direction of responses denoted as increasing (↑) or decreasing (↓). Details given if $p \leq 0.10$. 105

Table 5.1: Extent of change in air and soil parameters at roadsides based on data presented in chapters 2 and 3. M relates to soil moisture and n/a denotes that it is not appropriate to consider background concentrations for the stated factor. 122

Figure 1.1: Projected end-of-the-century (2080 – 2100) change in rainfall compared with present day in (a) winter (DJF) and (b) summer (JJA) in Europe (IPCC, 2008). 14

Figure 1.2: Geographical distribution of predicted above-ground biomass changes (AGB, g m^{-2}) in grassland ecosystems under 2050 predicted N. Regions represented as dark grey were not covered in the data set from which the model was constructed (Lee et al., 2010). 19

Figure 2.1: The negative logarithmic relationship between the mean concentrations of NO_2 ($\mu\text{g m}^{-3}$) and distance from the road (m) at the high TS (blue continuous line), medium TS (red dotted line) and low TS (green dashed line). Values represent means $\pm$ s.e. 52

Figure 2.2: The relationship between the log transformed distance from the road (m) and the monthly traffic volume on monthly concentrations of NO_2 ($\mu\text{g m}^{-3}$) at all three sites. This plane represents a fitted relationship using linear regression, with each point representing the mean of 3 diffusion tubes exposed for a month. Random effects used in the model were not applied to the relationship shown here, which is for illustrative purposes only. 53

Figure 2.3: The negative logarithmic relationships between soil Zn (black continuous line), Pb (grey dotted line) and Cu (orange dashed line) concentrations and distance from the road (m). Values represent means $\pm$ s.e across all three sites. 54

Figure 2.4: Relationship between pH and distance from the road in March 2010 at the high TS (blue continuous line), medium TS (red dotted line) and low TS (green dashed line). 56

Figure 3.1: Relationships between pH and road proximity ($p < 0.05$). Values represent means across seven sites $\pm$ s.e (SCH excluded). 80

Figure 3.2: Relationship between frequency-weighted Ellenberg N indicator scores and distance from the road ($p < 0.05$) as described by the LME model (black line). Site specific relationships are also given (see figure legend). 82

Figure 3.3: Relationship between frequency-weighted Ellenberg N indicator scores and modelled dry NO_2 deposition ($p < 0.001$) as described by the LME model (black line). Site specific relationships are also given (see figure legend). 83

Figure 4.1: Soil moisture (%) over time in June 2011 in the rainfall manipulated and ambient climate treatments. Values are the means of 3 measurements taken from each of the 8 rainfall manipulated and 8 ambient climate plots at each time point. The vertical line represents commencement of the summer treatments. * denotes $p < 0.05$. 106

Figure 4.2: Annual (a) mineralisation rates (b) nitrification rates across each of the four treatments. Values are the means of four replicates. Letters a and b denote significantly different treatments at $p < 0.05$. 107

Figure 4.3: Mean cover (%) of grasses and forbs in rainfall manipulated and ambient climate treatment in summer 2009, 2010 and 2011 without standard error bars for illustrative purposes. Values are means of eight replicates. † denotes near significance at $p \leq 0.1$. 109

Figure 4.4: Individuals (ind) of (a) *Auchenorrhyncha*, (b) *Aranae* and (c) *Hymenoptera* across each of the four treatments. Values are the means of four replicates. Letters a and b denote significantly different treatments at $p < 0.05$. 111

1. Introduction

Grassland ecosystems are dominated by rapidly growing plant species of low stature, extending across many regions of the world in arid, humid and temperate zones.

Natural grasslands cover 53 million square kilometres, around 40 % of the Earth's terrestrial surface (Suttie *et al.*, 2005). Approximately 34 % (650 – 850 Gt C) of the world's terrestrial carbon (C) is contained within these grasslands (White *et al.*, 2000) and they harbour important flora and fauna, with 35 of the 136 most species rich terrestrial eco-regions being designated as grasslands (White *et al.*, 2000). Over the most recent millennium, agricultural land has increased in cover to approximately 34 million square kilometres, around 26% of the Earth's surface, to feed the growing human population (Klein Goldewijk *et al.*, 2011). As crops and pasture of generally low conservation value have increased in abundance, natural grasslands have declined (Reidsma *et al.*, 2006). For example, natural grasslands have declined by 1,800 square kilometres across Europe during the 16 years from 1990 to 2006 with their composition and function becoming increasingly threatened by agricultural practises, road building, climate change, pollution and urbanisation (EEA, 2010). Some threats may have wide reaching consequences for the composition and functioning of grassland ecosystems, however, localised threats can lead to localised ecosystem responses. This thesis focuses on both global and local environmental changes, by assessing grassland responses to experimental manipulation of the nitrogen (N) cycle and climate, as well as investigating the suite of local environmental changes that can be brought about by roads.

1.1. Global environmental change

Global environmental change includes shifts in the composition of air, soils and water bodies, and changes to various aspects of the climate (IPCC, 2007). These changes will have important consequences for the composition (plant and animal species present) and functioning of ecosystems (ecosystem processes) in the future, because their flora and fauna will be presented with novel environmental conditions. The Millennium Ecosystem Assessment (2006) linked these ecosystem functions with ecosystems services, as “*functions or groups of functions carried out by ecosystems which improve human well-being*”. Pollination, climate regulation via carbon sequestration, food production, water purification, soil formation and maintenance of biodiversity are all examples of the services which can be provided by ecosystems. There is considerable uncertainty associated with predictions of whether grasslands will continue to deliver current levels of ecosystem services into the future. For example, modelled future annual grassland C sequestration rates range by 4 Gt C yr⁻¹, from grasslands acting as carbon sources (2 Gt C yr⁻¹) to grasslands acting as carbon sinks (-2 Gt C yr⁻¹) worldwide (Scurlock & Hall, 1998). This uncertainty is partially driven by insufficient quantitative data describing plant responses to the suite of anticipated global changes (Trumper *et al.*, 2009).

1.1.1. Climate change and grassland ecosystems

In the future the Earth's climate is expected to change, with climate models projecting that many of the world's ecosystems (particularly northern temperate zones) are at an increasing risk of experiencing frequent drought events (IPCC, 2007, IPCC, 2008). Across much of western Europe, it is anticipated that summer rainfall events will increase in intensity, but decrease in frequency leading to an overall decline in rainfall, whilst winter rainfall is projected to increase in both frequency and intensity (IPCC, 2008, Murphy *et al.*, 2009). For example, in the UK rainfall may increase by 30 % in the winter but decline by as much as 40 % in the summer (figure 1.1).

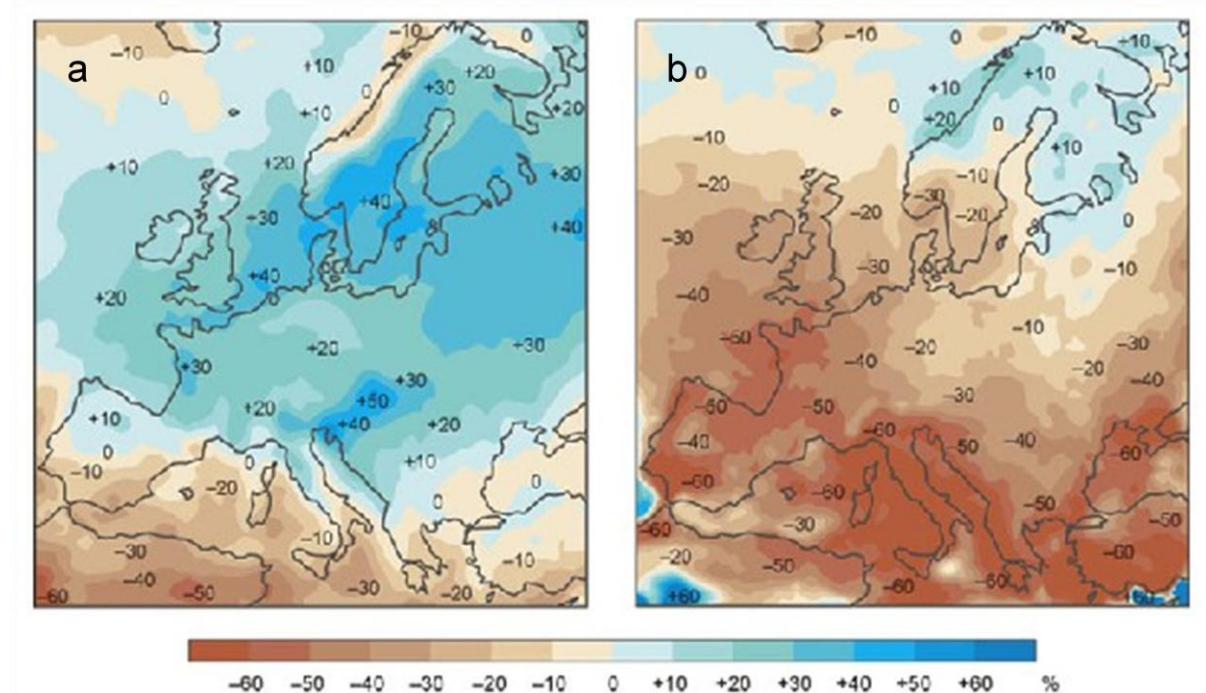


Figure 1.1: Projected end-of-the-century (2080 – 2100) change in rainfall compared with present day in (a) winter (DJF) and (b) summer (JJA) in Europe (IPCC, 2008).

Observations of grassland community responses to natural and experimental variation in rainfall regime have demonstrated that reduced plant biomass production (and hence C-sequestration), changes to species composition and localised species extinctions are symptomatic of exposure to drought and reduced soil moisture availability, though there is substantial variation between the responses of different grassland types (table 1.1). Variation in rainfall during the growing season may affect plant communities to the greatest extent, because some grassland communities are more responsive to environmental changes during this period (Suttle *et al.*, 2007). Comparison of annual net primary productivity (ANPP) responses in different ecosystems using 12 years of data from 11 North American research stations suggests that there may be a greater response in the ANPP of grasslands resulting from changes to the timing and magnitude of rainfall events than may be seen in other biomes (Knapp & Smith, 2001), with potential cascading effects on the services they provide.

Long-term above-ground biomass declines in response to drought have been frequently demonstrated, but the extent by which different grasslands are resistant or resilient to reduced moisture availability varies considerably (table 1.1). Rapid (< one year) biomass declines in response to extreme drought have been demonstrated (Tilman & Haddi, 1992), but adaptation of some grassland communities to variation in water availability means that they are able to maintain above-ground biomass and species diversity under drought conditions (Jentsch *et al.*, 2011). Other grasslands can suffer short term biomass losses but subsequently return to pre-drought productivity over-time (Grime *et al.*, 2000, Morecroft *et al.*, 2004, Evans *et al.*, 2011).

Table 1.1: Summary of plant composition and function responses to declines in annual rainfall (by manipulation of rainfall regime) in grassland ecosystems. Mean annual rainfall (MAR), mean average temperature (MAT) are also given.

Location	Grassland type	Drought treatment	MAR (MAT)	Duration (years)	Biomass change (above ground unless stated)	Composition or richness change
USA ^a	Upland grassland	40% decline	400 (8)	1	Decline in biomass in year of drought	Richness decline, reduction of grasses
UK ^b	Limestone grassland	0% reapplied July - August	1300 (8)	5	Decline in year 2, but not in subsequent 3 years	Decline in sedges
Canada ^c	Temperate grassland	100% re-applied	906 (4)	1	Decline as periods of drought (between irrigation) increased	Not measured
UK ^d	Limestone grassland	~90% decline	680 (10)	8	Decline in year 2, persisting for 5 years except in final year	Decline in grasses, increase in forbs
USA ^e	Prairie	30% decline	834 (8)	4	Decline over 4 years	Richness increase
Argentina ^f	Shortgrass steppe	30%, 55%, 80% decline	168 (8)	3	Decline in year 2, persisted for 1 year	Decline in grass, decline in richness
Czech Republic ^g	Lowland acid	50% decline	587 (9)	2	Decreased root growth in years 1 and 2	Not measured
	Highland acid		760 (6)	2	Decreased root growth in year 1 not 2	Not measured
	Mountain acid		947 (7)	2	Decreased root growth in year 1 not 2	Not measured
USA ^h	Shortgrass steppe	25% or 50% reapplied Apr - Oct	341 (8)	11	25% treatment: decline in 5th year persisted for remaining 6 years	Richness decline in years 2 - 8
					50% treatment: decline in only 5th and 11th years	Richness decline in years 2 - 4
Germany ⁱ	Mesotrophic grassland	100% decline Apr - Jun	741 (8)	5	No above- or below-ground drought effect in 5 years	None in 5 years

^a Tilman & Haddi, 1992, ^b Grime et al., 2000, ^c Laporte et al., 2002, ^d Morecroft et al., 2004, ^e Harper et al., 2005, ^f Yahdjian & Sala, 2006, ^g Fiala et al., 2009, ^h Evans et al., 2011, ⁱ Jentsch et al., 2011.

Grassland plant species which can efficiently uptake water (e.g. reducing transpiration) and/or those which invest a greater proportion of their resources below-ground are better adapted to extract scarce water in the soil, hence forbs and C4 grass species frequently increase in abundance in response to drought, whilst C3 grasses and sedges decline (Tilman & Haddi, 1992, Grime *et al.*, 2000, Morecroft *et al.*, 2004, Yahdjian & Sala, 2006). Such compensatory mechanisms may be more prevalent in species rich assemblages, where there is an increased probability of the presence of one or more species which can compensate for declines in other species (Yachi & Loreau, 1999). However, other mechanisms describing plant community resistance to drought have also been proposed including the extent of previous exposure to drought, functional composition and successional status (Grime *et al.*, 2000).

1.1.2. Nutrient cycling and grassland ecosystems

One of the key global changes that has taken place over the last 150 years is anthropogenic transformation of the N cycle (Galloway *et al.*, 2008). N₂ is relatively stable and inert in the atmosphere, but inputs of reactive oxidised and reduced N compounds (e.g. nitrogen dioxide and ammonia) can influence ecosystem composition, processes and services (Galloway *et al.*, 2004, Bobbink *et al.*, 2010). Reactive N creation has increased approximately 12 fold between 1860 and 2005, with total N creation reaching 187 Tg N yr⁻¹ in 2005, predominantly the result of increased energy production, industry, transport and agriculture (Galloway *et al.*, 2008).

The continued input of anthropogenic derived reactive N into the N cycle has led to shifts in the abundance and distribution of N globally, with N deposited from the air to

plants and soils at an increasing rate (Galloway *et al.*, 2008). Currently 11% of the world's natural vegetation receives N deposition inputs greater than $10 \text{ kg ha}^{-1} \text{ yr}^{-1}$, a deposition rate which would have been extremely rare in 1860 (Dentener *et al.*, 2006). N deposition rates are predicted to continue to rise, with substantial areas of the world projected to be exposed to N deposition rates greater than $50 \text{ kg ha}^{-1} \text{ yr}^{-1}$ by 2050 (Galloway *et al.*, 2004). European N emissions increased 10 fold over the last century however; N emissions are predicted to remain relatively constant in the coming century. hence model outputs indicate that around 20 % of European vegetation will receive N deposition inputs greater than $10 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in 2050 (Dentener *et al.*, 2006). Disruption of the N-cycle is of particular ecological interest because N limitation is widespread across the world's ecosystems, with the productivity of many temperate forests, tropical forests, temperate grasslands, tropical grasslands, wetlands and tundra limited by the availability of N (LeBauer & Treseder, 2008).

Increased N deposition has been demonstrated to cause compositional changes in grassland plant and associated invertebrate communities, through declines in regional and local biodiversity and by favouring species which are superior N competitors (Bobbink *et al.*, 1998, Stevens *et al.*, 2004, Stevens *et al.*, 2006, Eisenhauer *et al.*, 2011). A meta-analysis of grassland manipulation experiments around the world documented a mean increase in shoot growth and a mean reduction in root growth in response to N enrichment, perhaps reflecting shifts in the limiting plant resource from N below-ground to light above-ground (Lee *et al.*, 2010). Increased above-ground growth can have important implications for the productivity of grasslands and it is anticipated that future biomass production in grasslands at a global scale will increase as N creation and N deposition rates increase (figure 1.2).

Biomass gains may therefore represent an important trade-off in ecosystem services in grassland ecosystems between increased C stocks and crop yields but declining biodiversity.

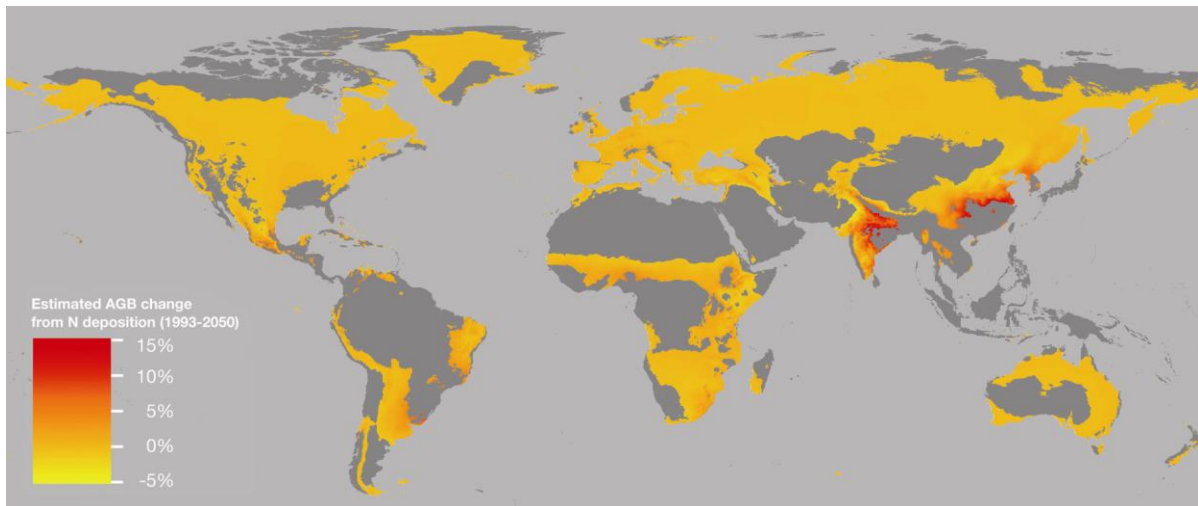


Figure 1.2: Geographical distribution of predicted above-ground biomass changes (AGB, g m^{-2}) in grassland ecosystems under 2050 predicted N. Regions represented as dark grey were not covered in the data set from which the model was constructed (Lee et al., 2010).

1.1.3. Interactions between nutrient cycling and climate

Meta-analyses of productivity responses to N enrichment across climatic gradients in individual grassland plant species and grassland plant communities have demonstrated co-limitation by rainfall and N availability (Hooper & Johnson, 1999, Xia & Wan, 2008). Plant productivity responses to reduced water and N availability can be more complex than simple additive effects. Addition of N can reduce the effects of low water availability on plant productivity in the short-term, because N uptake by roots is more water efficient under high N conditions (Tobar et al., 1994).

Resistance to drought and resilience following alleviation of drought have been demonstrated in crop plants under agricultural N loads (Tesda & Kumar, 1978, Radin *et al.*, 1985, Shangguan *et al.*, 2000). Lower plant community productivity gains in response to N enrichment have been found in regions experiencing low rainfall and lower background N deposition rates (Lee *et al.*, 2010). This more than additive interactive effect is perhaps driven by reduced mobility of N in dry soils since additional N is less readily washed over root surfaces.

Despite the demonstrated interaction between increased local rainfall, N enrichment and greater biomass production across grassland manipulation experiments, there are only a few studies which have investigated the responses of grasslands to N enrichment and manipulation of rainfall regime simultaneously in natural grasslands. One such study documented that above-ground productivity increases in a Californian grassland only occurred when additional rainfall and N were added together, indicating co-limitation by N and water (Harpole *et al.*, 2007). Another study demonstrated greater than additive biomass gains in response to water and N addition in a semi-arid grassland ecosystem (Lauenroth *et al.*, 1978). However, there are no studies in natural grasslands which have investigated the effects of both a reduction in rainfall and N enrichment simultaneously (Bobbink *et al.*, 2010). This has only been investigated in heathland, where N addition and drought together increases community susceptibility to invasion by grasses (Bobbink *et al.*, 1998).

Grassland community responses to N may also be influenced by other factors, for example some grassland ecosystems are co-limited by the availability of other essential plant nutrients including phosphorus (Wilson *et al.*, 1995, van der Hoek *et al.*, 2004) and potassium (Barger *et al.*, 2002). It has also been demonstrated that there are greater species losses in grassland plant communities exposed to N

enrichment when there are greater productivity increases, lower soil exchange capacities and colder regional temperatures (Clark *et al.*, 2007).

This thesis seeks to provide information to fill this knowledge gap by experimentally manipulating rainfall regimes to reduce overall annual rainfall and increase N deposition in a mesotrophic grassland ecosystem. The aim of this experiment is to investigate ecosystem compositional and functional responses to simulated end-of-century rainfall based on IPCC 4th assessment projections and increased N deposition (Galloway *et al.*, 2004, IPCC, 2007, Galloway *et al.*, 2008, IPCC, 2008, Murphy *et al.*, 2009). This study will also assess the degree to which the experimental grassland is limited by N, water or other essential plant nutrients. Data can be used to reduce the uncertainty surrounding predictions of future changes to ecosystem services in response to novel environmental conditions.

1.2. Environmental change: the contribution of roads and road vehicles

Roads are now estimated to extend around five million kilometres across Europe, four million kilometres across the USA, three million kilometres across China, and a million kilometres across Russia and Japan (ERF, 2010). The vehicles that use these roads emit many pollutants into the air, either via fuel combustion or from the wear and tear of brakes, engines and tyres. Pollutants include; carbon monoxide (CO), carbon dioxide (CO₂), oxides of nitrogen (NO_x), ammonia (NH₃), hydrocarbons (HC), volatile organic chemicals (VOCs), poly-aromatic hydrocarbons (PAH), heavy metals (HMs), nitrous acid (HONO) and methane (CH₄), with some pollutants also acting as precursors promoting formation of the secondary pollutant ozone (O₃) (Wallington *et al.*, 2008). The result of such an extensive worldwide road network is that vehicular traffic may contribute considerably to global environmental change.

For example, across Europe and USA road transport is estimated to contribute 22 % and 24 % of total CO₂ emissions respectively (IPCC, 2007); across Europe, road transport is the largest single contributor to overall emissions of oxidised N (RoTAP, 2011).

Roads and road vehicles can also have important effects locally; changing the composition of the air, the biogeochemistry of soils and also disrupting hydrology (Spellerberg, 1998). These environmental changes may have important effects on the composition and functioning of plant communities. In order to understand how environmental changes can also influence the structure and function of grassland ecosystems locally, the second part of this introduction focuses on the research that has been carried out on the ecological effects of roads and road vehicles.

1.2.1. Air quality alongside roads

Vehicles emit a cocktail of gases and particles (Wallington *et al.*, 2008). The extent by which these gases and particles are deposited to soils and vegetation at roadsides (dry deposition) is dependent upon their deposition velocity (V_d), the concentration at which they are emitted and the adsorptive properties of the surfaces themselves. Local environmental factors may also play a role, including the direction and strength of the prevailing wind, which may disperse airborne pollutants different distances from roadsides (Gilbert *et al.*, 2003). Physical barriers (e.g. walls, trees, buildings) and/or topographical features which can impede the dispersal of pollutants away from roads (Yin *et al.*, 2011) and chemical reactions between different exhaust products in the air are also important determinants of roadside pollutant concentrations. Some soluble exhaust products also dissolve in water and are deposited in rain (wet deposition).

Variation in the V_d of different vehicle-derived pollutants means that they persist at elevated concentrations different distances from a given roadside. The high V_d of HMs, for example, results in relatively rapid deposition from the air to soils and vegetation; hence elevated concentrations of HMs in the air do not persist as far from road edges as many exhaust gases (Table 1.2). Aerosol HM concentrations have been measured to decline to background levels approximately 20 to 30 m from busy roads (Little & Wiffen, 1978, Harrison & Johnston, 1985). Similarly, NH_3 also has a relatively high V_d , with NH_3 concentrations (1.5 m from the ground) measured to decline by 90 % within the first 10 m of road edges (Cape *et al.*, 2004).

Table 1.2: Dry deposition velocities (V_d) of exhaust gases from the air to plant surfaces. V_d for HMs is represented by Pb and is for indicative purposes.

Species	V_d (cm s ⁻¹)
CO_2	0.3 ^a
Ozone	0.5 ^a
NO	1 – 2 ^b
VOCs	2 – 3 ^c
NO_2	1 – 3 ^b
PAH	2 – 8 ^d
NH_3	10 – 19 ^b
HM (Pb)	33 ^e

^a Sehmel, 1980, ^b Hanson & Lindberg, 1991, ^c Su *et al.*, 2007, ^d Vardar *et al.*, 2002,

^e Fowler *et al.*, 2004

VOCs and PAHs are present alongside roads in gaseous and particulate phases. PAH particles can be deposited rapidly at the roadside, for example, particulate PAH concentrations were measured to decline to background levels within 4 m of a UK motorway (Harrison & Johnston, 1985). Gaseous PAHs have intermediate V_d , with roadside PAH concentrations estimated to decline to background concentrations 60 – 100 m from road edges, based on measurement of PAH accumulation in bryophytes (Viskari *et al.*, 1997). NO also has an intermediate V_d , but oxidation of NO to NO₂ means that NO reduces to background concentrations at a similar rate to NH₃ (Cape *et al.*, 2004). The relatively low V_d of NO₂ and oxidation of NO to NO₂ means that elevated concentrations of NO₂ have been measured up to 1 km from roadsides (table 1.3). Low road derived inputs of CO₂ relative to ambient (background) concentrations and its low V_d means that the local ecological effects of CO₂ are likely to be minor (Signal *et al.*, 2004).

Table 1.3: Summary of studies measuring air quality at different distances perpendicular to roadsides including the traffic densities of the roads that the study considered, the distance from the road that background concentrations were reached and the maximum concentrations (c) of NO₂ and NH₃ measured at roadsides. Na refers to unmeasured variables.

Location	Site		NO ₂ (µg m ⁻³)		NH ₃ (µg m ⁻³)	
	Traffic (million veh yr ⁻¹)	Transect Length (m)	Max c roadside	Distance (m) to background	Max c roadside	Distance (m) to background
Wales ^a	0.01 - 2	250	37	~ 150	na	na
Denmark ^b	9	1,000	31	> 1,000	na	na
Germany ^c	23 - 37	520	na*	~ 230 m	na	na
Holland ^d	29 - 55	300	57	~ 150	na	na
England ^e	37	~275	48	~ 100	na	na
Canada ^f	52	1310	56	> 1,000	na	na
USA ^g	73	388	23	> 400	na	na
Scotland ^h	21 - 88	10	41	> 10	6	> 10

^a Bell & Ashenden, 1997, ^b Glasius et al., 1999, ^c Bernhardt-Romermann et al., 2007,

^d Roorda-Knape et al., 1999, ^e Bignal et al., 2007, ^f Gilbert et al., 2003, ^g Rodes & Holland, 1981, ^h Cape et al., 2004.

Air pollution research has driven policy changes around the world, with local governments in the UK required to monitor a number of pollutants. Critical levels (CLEs), referring to airborne pollutant concentrations (table 1.4) have been established to protect ecosystems from pollution damage (WHO, 2000, DEFRA, 2007). There are also three EU directives that relate directly to air quality; the First Air Quality Daughter Directive (1999/30/EC) which focuses on annual average NO₂, the National Emissions Ceilings Directive (2001/81/EC) which sets a ceiling on the emissions of a number of pollutants (including NO_x for each member state) and the Third Air Quality Daughter Directive (2002/3/EC) which presents a target for O₃ concentrations but requires monitoring of other pollutants. There is currently no EU directive for monitoring of NH₃, however, there is growing evidence of the negative environmental influences of elevated NH₃ concentrations in terms of reduced plant biodiversity and vitality across several ecosystem types (Van Der Eerden *et al.*, 1991, Erisman *et al.*, 2008, Cape *et al.*, 2009, Sheppard *et al.*, 2009, Bobbink *et al.*, 2010, Sheppard *et al.*, 2011).

Table 1.4: Summary of air quality targets for the protection of vegetation and ecosystems in the UK (adapted from DEFRA, 2007). AOT40 = sum of the excess hourly concentrations greater than 80 µg m⁻³ (40 ppb).

Pollutant	Critical level	Measurement metric
NO _x	30 µg m ⁻³	Annual mean
NH ₃	8 µg m ⁻³	Annual mean
SO ₂	20 µg m ⁻³ 20 µg m ⁻³	Annual mean Winter average (Oct – Mar)
O ₃	18,000 µg m ⁻³ h ⁻¹ averaged over 5 years	AOT40, calculated from 1 hour values (May – July)

1.2.2. Soil biogeochemistry changes at the roadside

Roads and road vehicle effects on the surrounding environment are not limited to changing air quality. Simulated road construction has demonstrated the potential for roads to alter soil hydrology (Tague & Band, 2001). It has been shown that roads can induce enhanced surface run-off (Luce & Cundy, 1994) because roads are only partially impermeable to water with between 6 and 9% of water infiltrating through modern road surfaces (Ragab *et al.*, 2003). The run-off of rainfall from road surfaces to adjacent soils can increase soil moisture (Boivin *et al.*, 2008), however, roads running through forests can reduce soil moisture if changes to the vegetation promotes desiccation of soils (Marsh & Beckman, 2004).

Road run-off can also carry dissolved and suspended road surface chemicals, depositing them at roadsides (Backstrom *et al.*, 2003). Salt is applied to many roads to ameliorate traffic disruption as a result of snow and ice, with a solution of NaCl and CaCl_2 common de-icing agents. Salt application can lead to reduced ammonium (NH_4^+) retention and increased mineralisation rates in roadside soils (Green & Cresser, 2008). Soils of higher pH have been found nearer to roads in Germany, where a solution of CaCl_2 (pH-value: 8.5 – 9.5) was used as detergent to enhance the efficacy of road salt (Bernhardt-Romermann *et al.*, 2007). Other road surface chemicals, particularly those applied to roads during road maintenance, can also be transported in solution to the roadside raising soil pH (DfT, 2008).

A brine solution comprising 30% salt and 70% water is applied onto roads in the UK throughout the winter (S.Mitchell, *pers comm*). A transect study of roadside soils documented an increase in concentrations of sodium in soils up to 20 m to 30 m from busy roads in Italy during the winter, however, these high concentrations were

only found in the winter as the soluble salt was leached from soils following rainfall (Viskari *et al.*, 1997). Salt concentrations in soil have also been shown to be elevated up to 150 m from a major road in Denmark, with peak concentrations measured in the winter when salt application is at its maximum (Pedersen *et al.*, 2000). Where salt is used less frequently (e.g. UK) elevated salt concentrations are associated with fast moving traffic, accumulating in central reservations and roadside verges but declining rapidly; measurements alongside a busy motorway indicate that soil salt concentrations decline by 70%, between 2 m and 4.5 m from a busy road (Thompson *et al.*, 1986).

HMs from vehicle exhausts, degradation of brakes and tyres and erosion of the road surface itself can be deposited to roadside soils in run-off (Backstrom *et al.*, 2003) and airborne dust (Wang *et al.*, 2007a). This means that there are two pathways for HMs to reach nearby ecosystems. Titanium (Ti), chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) can be elevated in soils and/or plant tissues at roadsides (Daniel *et al.*, 1997). Bryophytes are very sensitive to changes in HM concentration, and elevated tissue concentrations of several HMs have been measured up to 1,000 m away from busy roads (Zechmeister *et al.*, 2005). More commonly, however, HMs will only be elevated near roadsides. For example soil concentrations of Pb, Cu, Cd and Zn have been measured to decline by 82 %, 99 %, 83 % and 95 %, respectively, within 5 m of busy roads (Pagotto *et al.*, 2001).

1.2.3. Response of grassland plants to pollutant exposure

There have been numerous studies investigating the responses of grassland plants to pollutant exposure. Above-ground biomass of the grass *Poa pratensis* increased in the first four months of fumigation with NO₂ at 115 µg m⁻³. However, growth rates declined in the NO₂ exposed plants in the following six months of the study, resulting in no difference in the biomass of treatment and control plants after 11 months (Whitmore & Freersmith, 1982). Leaf area and above-ground biomass production were reduced in the same grass species exposed to NO₂ at 140 µg m⁻³ for one year, however growth rates of the grass *Dactylis glomerata* did not vary in response to the NO₂ treatment over the experimental period (Ashenden, 1979). Growth rates of two other grass species *Lolium multiflorum* and *Phleum pratense* did not change in response to exposure to NO₂ at 140 µg m⁻³ over a short 20 week study (Ashenden & Williams, 1980). Above ground biomass production in *Carex nigra* and *Carex alba* declined over a six week period in response to 281 µg m⁻³ of NO₂, whilst not significant, there was a trend towards reduced above-ground biomass production across five further urban grassland species (*Sonchus oleraceus*, *Lolium perenne*, *Poa annua*, *Trifolium repens*, *Rumex acetosa*) in response to fumigation with NO₂ over the same period and at the same dosage (Bell *et al.*, 2011).

Tissue N in plants exposed to NO_x can be increased due to uptake via stomata and assimilation of NO_x under the control of the enzymes nitrite reductase and nitrate reductase. Rates of reaction in these enzymes have been demonstrated to be increased in *Dactylis glomerata*, *Lolium multiflorum* and *Phleum pratense* within a week of exposure to 140 µg m⁻³ of NO₂, continuing throughout a 20 week study (Wellburn *et al.*, 1981). Rowland *et al.* (1987) demonstrated that increased

assimilation of NO₂ from the air into *Hordeum vulgare* tissue only occurred when soil N was low, due to increased activity of nitrite reductase and nitrate reductase in N deficient plants.

Many single pollutant fumigation studies have exposed grassland plant to elevated concentrations of NO_x but few have assessed the effects of the other exhaust emissions. The grass *Lolium perenne* was shown to assimilate benzene and toluene into plant tissue when exposed to four VOCs at approximately 250 ng g⁻¹ (Binnie *et al.*, 2002). *Deschampsia flexuosa* can assimilate NH₃ into plant tissue with much of the additional N uptake via roots rather than leaf adsorption (Dueck *et al.*, 1991). NH₃ concentrations of 0.02 µg m⁻³ have been demonstrated to increase productivity in *Zea mays*, however increasing NH₃ concentrations to 2 µg m⁻³ was demonstrated to reduce productivity (Zhang *et al.*, 2011). Roadside elevated ozone concentrations may be short-lived, however, visible injuries have been observed in *Dactylis glomerata*, *Dactylis aschersoniana* and *Phleum alpinum* in response to exposure to elevated ozone concentrations for one month, with no effects of treatments on relative growth rates (Pleijel & Danielsson, 1997). The ecological consequences of many of these plant responses to single pollutants are not well resolved and very few studies have investigated the effects of more than one pollutant.

Viskari (2000) observed increased damage to epicuticular waxes and mesophyll in *Picea abies* following 10 days of exposure to exhaust gases in a controlled fumigation study, attributing these changes to NO_x and VOCs (mean NO_x concentration: 26 to 104 µg m⁻³). Observations of the responses of herbaceous plants to a realistic mix of diesel exhaust emissions using a solar dome fumigation facility (annual mean NO_x ranged from 77 to 98 µg m⁻³) identified species-specific changes to phenology in response to vehicle exhaust pollution, with a trend toward

increased senescence and delayed flowering (Honour *et al.*, 2009). Analyses of growth responses following exposure to the same diesel fumigation regime showed species-specific changes to growth rates, with the productivity of some plant species (e.g. *Poa pratensis*) increased whilst the productivity of other species (e.g. *Carex nigra*) declined (Bell *et al.*, 2011).

Transplanted *Ricinus communis* plants grown in pots, 5 metres from a busy road, had greater leaf chlorophyll content and total N after 5 months of exposure to exhaust gases (Kammerbauer & Dick, 2000). Similarly, total tissue N content and leaf N content increases in transplanted *Molinia caerulea* growing in soil cores at different distances from roadsides were associated with increases in NO_x concentrations (Laffray *et al.*, 2010). Species-specific responses to fumigation with vehicle exhaust products suggests that shifts in competition between species, with some species favoured over others in the roadside environment, may influence the composition of roadside plant communities and that N inputs are likely to play an important role.

1.2.4. Responses of grassland plant to HM enrichment

Uptake of HMs is primarily via the roots, hence fumigation studies are not appropriate for testing grassland plant responses to HMs (Bignal *et al.*, 2004). Some HMs are essential for plant growth and development (e.g. Cu and Zn) and are found naturally in soils (Graham, 1981, Cakmak *et al.*, 1997, Pilon *et al.*, 2006). Ni, Cr and Cd concentrations in cereal crops have been demonstrated to increase nearer roadsides in response to elevated HM inputs (Antanaitis *et al.*, 2007). Increasing Cu has been demonstrated to increase productivity in wheat crops in Cu deficient soils (King, 1974). However, at high concentrations some HMs can be toxic to plants

(Ross & Kaye, 1994). For example, adding 2,000 mg l⁻¹ of Pb to soils reduced above-ground biomass accumulation in *Lythrum salicaria* (Uveges *et al.*, 2002).

1.2.5. *In-situ* effects on vegetation

In-situ studies can demonstrate the effects of the suite of roadside environmental perturbations on the structure and functioning of roadside plant communities, with roadside edge effects on plant communities extending beyond 200 m from roadsides (Bernhardt-Romermann *et al.*, 2007). Angold (1997) used a transect study to assess the effects of adjacent roads on dry heathland in the New Forest, UK (35,000 vehicles counted in a 12 hour period), in which changes to species composition, from *Calluna vulgaris* dominated heathland farther from the road to a *Molinia caerulea* dominated grassland nearer to the road, were associated with N inputs from vehicle exhausts. Bernhardt-Römermann *et al.*, (2006) also used a transect study to correlate increased abundances of N and pH tolerant plant species alongside roads with NO₂ emissions and basic substances used to increase the efficacy of road salt in the forest floor vegetation of Forstenrieder Park and Hofoldingner Forest, Germany (23 and 38 million vehicles per year respectively).

Signal *et al.*, (2007) observed increases in the abundance of species adapted to high N conditions at a blanket bog at Moss Moor, UK. The study was, however, unable to decouple the influences of multiple roadside environmental perturbations on the declines in oak tree health alongside other busy roads. Ruderal, disturbance-tolerant, and salt-tolerant plant species have also been demonstrated to increase in roadside verges as the result of vehicular (wind) disturbance and the deposition of salt dissolved in road run-off (Truscott *et al.*, 2005). Alongside a busy city road, increased growth rates of *Trifolium repens* and degradation of leaf surface waxes in

Rumex acetosa and *Poa pratensis* were also associated with elevated NO and NO₂ concentrations from roads (Bell *et al.*, 2011).

The key limitation of many *in-situ* studies is that they represent a trade-off between the number of sites and the number of environmental variables measured (table 1.5). Field and fumigation studies have shown that many different exhaust products and environmental changes brought about by roads may influence plant communities growing alongside them. Studies frequently measure a limited number of environmental variables and hence disentangling the effects of many different roadside perturbations is problematic. This thesis seeks to address this by comparing a suite of roadside environmental variables with plant community composition for the first time in species rich calcareous grasslands.

Table 1.5: Summary of transect studies in ecosystems perpendicular to roadsides identifying which environmental variables were measured (x) and correlated (*) with plant species composition or foliar chemistry in each study. Effect zone represents the stated zone of roadside influence. Soil parameters: extractable (ext) N, heavy metal concentrations (HM), total soil carbon (C) and total nitrogen content (N).

Study system	Transect length	n	Air and rainfall			Soil					
			dry NO ₂	dry NH ₃	wet N	Ext N	Salinity	C	N	HM	pH
Heathland ^a	200 m	14	*								
Blanket bog ^b	365 m	1	x*								
Roadside verge ^c	10 m	92	x	x			*				
Forest ground flora ^d	520 m	2	x*	x	x	x*	x	x	x	x	x*

^a Angold, 1997, ^b Bignal *et al.*, 2007, ^c Truscott *et al.*, 2005, ^d Bell *et al.*, 2011, ^e

Bernhardt-Romermann *et al.*, 2007.

1.3. Calcareous grasslands as roadside study systems

Calcareous grasslands are among the most species rich habitats in Western Europe (Hodgson *et al.*, 2005). The plant community present is often dictated by soil nutrient availability with communities of high conservation value being associated with a narrow range of ecological conditions including alkaline soil, low soil fertility and frequent drought events (Critchley *et al.*, 2002, Bennie *et al.*, 2006). Calcareous grassland and roadside verges supporting calcareous plant communities are priority habitats for the protection of biodiversity in the UK (UKBAP, 2007) and across Europe (Pedro Silva *et al.*, 2008). Despite their high conservation value, calcareous grasslands have declined by 18,000 ha in the UK in the period from 1984 to 2007 (Countryside-Survey, 2008). Local extinction of rare species has been documented and invasion by more common species threatens to further reduce the abundance of calcareous grassland plants (Bennie *et al.*, 2006).

N enrichment of 20 kg ha⁻¹ yr⁻¹ and 80 kg ha⁻¹ yr⁻¹ has been demonstrated to increase calcareous grassland productivity, increasing the biomass of the forbs; *Filipendula vulgaris*, *Scabiosa columbaria* and *Thymus polytrichus* (Wilson *et al.*, 1995). However, no plant growth responses or compositional changes were seen in calcareous grasslands in response to N enrichment of between 35 kg ha⁻¹ yr⁻¹ and 140 kg ha⁻¹ yr⁻¹ elsewhere, despite elevated nitrification rates and soil acidification at the highest N doses (Carroll *et al.*, 2003). Critical loads for N deposition have been set in calcareous grasslands (and other ecosystems types) as benchmark values above which ecological changes are anticipated (Table 1.6).

Table 1.6: Summary of N critical loads in grassland ecosystems (Bobbink & Hattelingh, 2011). Critical loads relate to the maximum deposition of N that an ecosystem is able to sustain without harmful effects.

Ecosystem	Critical load (kg N ha ⁻¹ yr ⁻¹)	Indicator
Calcareous grassland	15 – 25	Increased tall grasses, decline in diversity, increased mineralisation, N leaching
Neutral-acid grassland	10 – 20	Increase in grasses, decline in some species
Montane-subalpine grassland	5 – 10	Increase in nitrophilous grasses, reduced diversity
Dry heathland	10 – 20	Transition from heather to grass, decline in lichen

Plant compositional changes in calcareous grasslands have also been seen in response to changes to grassland management, with grazing, mowing and burning treatments favouring different calcareous assemblages (Kahmen *et al.*, 2002, Moog *et al.*, 2002). Calcareous grassland communities are an important study system for identifying changes to the composition and functioning of the plant communities present as they are of high conservation value. Low nutrient status and exposure to frequent drought events may mean that calcareous grasslands are particularly susceptible to the suite of environmental perturbations associated with roads.

1.4. Aims and objectives

1.4.1. Road effects on grassland ecosystems

- Select study sites, gain permissions from landowners/managers and establish monitoring studies aimed at measuring road-derived environmental changes in calcareous grasslands.
- Quantify the extent to which roads can change air quality, soil biogeochemistry, plant composition and plant productivity at calcareous grasslands.
- Establish which environmental drivers are the key determinants of plant compositional and functional changes at roadsides.
- Identify individual plant species or groups of plant species which are favoured at roadsides.
- Highlight management regimes which may reduce the effects of road derived environmental changes in calcareous grasslands.

Hypotheses are: (1). Road run-off increases soil moisture content at roadsides carrying suspended HMs and salts to roadside soil. (2). Plant compositional changes reflect environmental changes at roadsides favouring plant species or groups of plant species which are adapted to roadside conditions. (3). N enrichment, derived from vehicle exhausts, is a key driver of changes in plant community composition at roadsides. (4). Roadside conditions favour species which are not typical calcareous species due to increased nutrient availability and reduced severity of drought conditions.

1.4.2. Climate and N effects on grassland ecosystems

- Design and carry out a long-term manipulation experiment simulating future rainfall regimes and nitrogen enrichment in a grassland ecosystem.
- Quantify soil biogeochemical responses to N enrichment and rainfall manipulation.
- Determine whether productivity in the experimental grassland is limited by the availability of N and/or water.
- Compare plant and arthropod compositional and functional responses to N enrichment and rainfall manipulation.
- Establish whether N enrichment and manipulation of rainfall regime applied together will interact to modify edaphic, arthropod and plant community responses to N and rainfall regime on their own.

Hypotheses are: (1) Soil moisture content, plant productivity, herbivorous insects and their predators will decline in climate change plots in response to summer drought, with increases in the abundance of drought tolerant plant species. (2) Soil N availability and plant productivity will increase in N enriched plots with associated increases in N tolerant plant and arthropod species. (3) Plant biomass declines in response to climate change will be reduced or prevented by N enrichment when both climate change and N enrichment are simulated in combination, hence avoiding plant and arthropod community shifts.

1.5. Thesis outline

Chapter 2 describes a detailed study of air quality, soil biogeochemistry, plant species composition and plant biomass production in relation to roadside proximity at three calcareous grassland sites immediately alongside a high, medium and low trafficked road. Relationships between air quality, soil biogeochemistry and roadside proximity were identified. Analyses established which of the measured environmental factors influenced plant species composition, species richness and community biomass production at each site.

Chapter 3 expands on the results from chapter 2 to develop a broader study of the effects of roads on plant composition in a larger number (eight) of calcareous grassland ecosystems. This multi-site approach allowed the use of mixed effects models which tested the ubiquity of the relationships identified in chapter 2 and assessed which plant species were increasing or decreasing at the roadside. Measurement of multiple environmental variables coupled with modelled NO_2 and NH_3 deposition identified likely key drivers of observed changes.

Chapter 4 presents an experiment aimed at furthering our understanding of how grasslands may respond to novel combinations of increased nitrogen deposition and changing climate in the future, by simulating both predicted future rainfall patterns and increased N deposition at a grassland site. Shifts in species diversity of the plant and arthropod community were monitored, in addition to above- and below-ground plant biomass and soil biogeochemistry.

Chapter 5 is the concluding chapter in which the overall findings are summarised. Data are discussed with reference to how they may contribute to our understanding of air pollution and climate change effects on the structure and functioning of grassland ecosystems. Management techniques which may prevent further changes brought about by roads to nearby calcareous grassland ecosystems are discussed. Mechanisms for understanding grassland resistance and resilience to climate change are also discussed. Recommendations are made for future work that will extend our knowledge in these areas.

The following chapter has been published in the
journal **Environmental Pollution**

LEE M., DAVIES L., POWER S. (2012) Effects of roads on adjacent plant community composition and ecosystem function: an example from three calcareous ecosystems. *Environmental Pollution*, **163**, 273-280.

2. Effects of roads on adjacent plant community composition and ecosystem function: an example from three calcareous ecosystems

2.1. Abstract

Roads and exhaust emissions can affect plant communities directly, for example via direct foliar uptake of exhaust products, or indirectly via changes to soil biogeochemistry and hydrology. A transect study adjacent to roads of different traffic densities was carried out at three species-rich calcareous grasslands in south eastern England. Measured annual NO_2 concentrations and modelled NH_3 concentrations increased towards the roads and with higher traffic densities, and there was evidence of increased soil moisture, pH and heavy metal concentrations at roadsides. Increases in the abundance of nitrogen (N) tolerant species and grasses at roadsides were associated with N enrichment from vehicle exhausts at two of the sites. In contrast plant species richness, the abundance of forb and moss species declined at roadside locations. As vehicle usage spreads across the world, it is increasingly important to understand the effects of road traffic on adjacent ecosystems to inform traffic and conservation management policies.

2.2. Introduction

Calcareous grasslands are of high conservation importance because they support a diverse range of plants and insects, including many rare species. Over the last 60 years, the coverage of calcareous grasslands has declined, making way for increased agricultural land, roads and urban development across Western Europe (Hodgson *et al.*, 2005). The remaining calcareous grasslands are located in a narrow range of environmental conditions, predominantly in soils with low nutrient status and high pH (Critchley *et al.*, 2002) and are particularly susceptible to anthropogenic influences. Local extinction of rare species has been documented and invasion by more common species threatens to further reduce the abundance of calcareous grassland plants (Bennie *et al.*, 2006).

Across Great Britain, 34 million vehicles travelled over 496 billion kilometres in 2010. 17% of these kilometres were travelled within the densely populated South East region of England (DfT, 2011) which itself harbours 20% of the 40,600 ha of UK lowland calcareous grassland (UKBAP, 1994). A consequence of this extensive road network is that vehicles are a key source of anthropogenic reactive nitrogen (N) in the area; despite the introduction of 3 way catalytic converters in Great Britain in 1992, motor vehicles remain the largest source of national emissions of oxides of nitrogen, contributing 30% of total emissions and also 2% of total ammonia (NH₃) emissions in 2007 (RoTAP, 2011). Across Europe, emissions of oxidised N declined by approximately 35% between 1990 and 2005, but road transport continues to be the largest single contributor to overall emissions (RoTAP, 2011).

In addition to N, motor vehicles also emit carbon dioxide (CO₂), carbon monoxide (CO), methane (CH₄), polyaromatic hydrocarbons (PAHs), particulate matter (PM), heavy metals (HM), volatile organic chemicals (VOC) and contribute to the formation of the secondary pollutant ozone (O₃) (Wallington *et al.*, 2008). The majority of these pollutants are emitted from car exhausts but PM and HM are also produced from the wear of engines, brakes and tyres. Roads can also change other environmental factors: Basic compounds are applied to roads which can influence pH and salt concentrations locally (Bernhardt-Romermann *et al.*, 2007); road surfaces themselves decay and also impede drainage (Ragab *et al.*, 2003); and higher levels of physical disturbance (wind turbulence) are associated with increasing rates of traffic flow (Truscott *et al.*, 2005).

Exhaust emissions are estimated to affect 5.4% of the area of Sites of Special Scientific Interest (SSSIs) in England i.e. they are situated within 200 m of a major road (Weigert, 2004). If a similar proportion of National Nature Reserves (NNRs) and Local Nature Reserves (LNRs) are also located in close proximity to roads, the total land area of sites of nature conservation value within the region under the influence of roads and vehicle exhaust emissions may be substantial, with significant consequences for the structure and functioning of semi-natural ecosystems.

Controlled fumigation experiments have demonstrated species-specific changes to plant phenology in response to petrol (Kammerbauer & Dick, 2000, Viskari *et al.*, 2000) and diesel emissions (Honour *et al.*, 2009), such as variation in growth rate, flower development and leaf senescence. In the field, plant compositional changes have been reported from a number of studies. These compositional changes include shifts in dominance from *Calluna vulgaris* to *Molinia caerulea* in UK heathland with increasing roadside proximity (35,000 vehicles counted in a 12 hour period),

attributed to elevated N derived from vehicles (Angold, 1997). Changes to the ground flora of two coniferous forests in Germany nearer the roadside (23.4 and 37.7 million vehicles per year) were linked with basic compounds used to enhance the efficacy of de-icing salt to the road and elevated atmospheric NO₂ concentrations (Bernhardt-Romermann *et al.*, 2007). Similarly, increases in the abundance of N tolerant species in a UK blanket bog were also associated with roadside pollution, represented by NO₂ increases at the roadside, although the effects of elevated NH₃ and other environmental roadside perturbations could not be discounted (Bignal *et al.*, 2007).

Plant compositional changes in calcareous grasslands have been observed in response to management (Kahmen *et al.*, 2002, Moog *et al.*, 2002), N enrichment (Willems *et al.*, 1993, Carroll *et al.*, 2003) and habitat fragmentation (Fischer & Stocklin, 1997). In order to determine how roads affect the composition and functioning of calcareous grassland ecosystems, a transect approach has been used to assess air quality, plant species composition, biomass production and soil biogeochemistry in relation to roadside proximity at three calcareous grassland sites immediately alongside a high trafficked site (high TS), a medium trafficked site (medium TS) and a low trafficked site (low TS) in the UK.

2.3. Material and methods

2.3.1. Study sites

Three grassland sites of high conservation importance were selected alongside roads of varying traffic densities in SE England (Table 2.1). Each site contained calcareous plant species growing on a chalk substrate, classified as CG2 and CG3 communities, with patches of mesotrophic grassland (Rodwell, 1992). The sites were: Aston Rowant National Nature Reserve (NNR), adjacent to the busiest road (high TS), the M40 motorway (51.678N, 0.943W); Butser Hill NNR, adjacent to a moderately busy road (medium TS), the A3 (50.973N, 0.974W); and Martins Down NNR, adjacent to a relatively quiet road (low TS), the A354 (50.980N, 1.949W). All three sites are managed using low intensity sheep grazing focussing on the restoration of plant species typical of calcareous grasslands.

Table 2.1: A summary of the sites used in this study including approximate road completion date and the distance (m) from the edge of the road to the first location marker. Annual vehicle density relates to mean annual vehicle numbers obtained from the nearest traffic count point over the ten years ending December 2008 (DfT, 2011). Traffic density is in million vehicles.

Site and designation	Road (completion date)	Traffic lanes	Distance from road edge	Annual Traffic density	Maximum monthly traffic density	Minimum monthly traffic density	Grazing Intensity (sheep ha ⁻¹)	Site brought under conservation management
Aston Rowant NNR	M40 (1974) ^a	6	7	35.0	5.6	2.7	2.5 – 2.7 ^a	1967 ^a
Butser Hill NNR	A3 (1979) ^b	4	10	17.1	1.5	1.3	1.1 – 2.1 ^b	1966 ^b
Martins Down NNR	A354 (1965) ^c	2	1	2.4	0.4	0.2	0.9 – 1.9 ^c	1978 ^c

^aR.Silverwood, *pers comm*, ^bT.Speller, *pers comm*, ^cL.Smith, *pers comm*.

2.3.2. Sampling technique

Three parallel transects 60 – 80 m apart were established at each of the three study sites, with a greater sampling effort taking place nearer the roadside following the study of Cape *et al.* (2004) which identified a 90% decline in NO₂ within 15 m of the road edge. Transects began as near to the road as possible within each NNR and then continued a further 2.5, 5, 7.5, 10, 20, 50, 100, 200 and 350 m (limited to 300 m at the high TS). There were therefore 30 sampling locations at each of the three sites making a total of 90 sampling sites. There was no known heterogeneity in historical land use or management within or between the three transects at each site.

2.3.3. Air quality

NO₂ concentrations were measured using Palmes diffusion tubes (7.1 cm long, diameter 1.1 cm) which were deployed in triplicate at 10 distance markers along one transect at all sites (Palmes *et al.*, 1976). The absorbent medium contained within each tube was 2,2',2''-nitriiotriethanol (C₂H₄O₃N), which has a high capacity to remove NO₂ from the sampled air (for further details on tube preparation see Palmes *et al.*, 1976, Cape., 2009). Tubes were positioned on wooden posts 75 cm from the ground to approximate maximum canopy height. The monitoring period was from July 2009 (October 2009 at the medium TS) to June 2010 inclusive, with tubes replaced monthly.

NO₂ absorbance by each tube, and hence mean NO₂ concentrations were determined using high performance liquid chromatography (Dionex, USA) by comparison with prepared standards. Travel blanks and re-analysis of 5% subsamples were used to ensure the accuracy of analytical methods. It should be noted that samples taken nearest the roadside from all three sites were at approximately the same elevation as the road which reduced height-induced

variation. NH_3 concentrations were modelled assuming a ratio of $\text{NO}_2:\text{NH}_3$ of 5:1 of the vehicular input NO_2 (i.e. above background concentrations) at the roadside, and were modelled to decay according to formula 1, where D is distance from the roadside (m), C_{bkgd} is the background concentration of NH_3 (DEFRA, 2011) and C_{veh} is the estimated concentration of NH_3 nearest the road (Cape *et al.*, 2004). The influence of the prevailing wind, which has been found to be significant in a study elsewhere (Gilbert *et al.*, 2003), was not considered.

$$(1) \text{NH}_3 = C_{\text{bkgd}} + C_{\text{veh}}e^{-0.25D}$$

2.3.4. Heavy metals in the soil

Soil samples were collected at the 30 locations within each site using 2.5 cm diameter and 15 cm length polyvinyl chloride (PVC) tubes in March 2010. Samples were homogenised and dried in a drying oven ($80\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$) for 48 hours. 1 g ($\pm 0.01\text{ g}$) sub samples were refluxed with 1:1 65% nitric acid and deionised water, 30% hydrogen peroxide and hydrochloric acid at $95\text{ }^{\circ}\text{C}$ (for further details on acid digestion methodology see van der Wiel, 2003). HM content was determined using inductively coupled plasma atomic emission spectrometry (ICP AES, Agilent Technologies, UK). Metals measured were lead (Pb), copper (Cu), zinc (Zn), vanadium (V), nickel (Ni), chromium (Cr), cobalt (Co), cadmium (Cd), arsenic (As), antimony (Sb) and molybdenum (Mo).

2.3.5. Soil pH and moisture

Soil pH was measured at all 30 sampling locations at each site in March 2010, May 2010 and August 2010 using a PHH-200 pH probe (Omega, UK). Soil moisture was measured using a ThetaProbe soil moisture sensor (Delta-T, UK) in the same

sampling periods. Both soil moisture and pH were measured at 5 cm soil depths with five replicates at each sampling location.

2.3.6. Extractable nitrogen and mineralisation

PVC cores (2.5 cm diameter, 15 cm length) were used to collect soil samples at 4 time intervals; June 2009, September 2009, December 2009 and March 2010, at each sampling location. PVC cores were then reintroduced into the ground adjacent to the original sampling location, covered to prevent leaching losses and left for three months before the soil was collected (incubated sample). Inorganic oxidised nitrogen ($\text{NO}_2^- + \text{NO}_3^-$) and reduced nitrogen (NH_4^+) were extracted from a 10 g sub-sample of incubated and fresh soil by mixing with 37.5 ml of 1 M KCl and concentrations were measured using a SKALAR San Flow ++ auto-analyser (SKALAR, Netherlands).

Mineralisation rates (MR) and nitrification rates (NR) were calculated for summer (June 2009 – September 2009), autumn (September 2009 – December 2009), winter (December 2009 – March 2010) and spring (March 2010 – June 2010) by comparison of extractable N content of incubated (t_2) and fresh soil samples (t_1) using formulae 2 and 3 below:

$$(2) \text{ MR} = \text{total extractable N } (t_2) - \text{total extractable N } (t_1) * \text{bulk density, and}$$

$$(3) \text{ NR} = \text{extractable oxidised N } (t_2) - \text{extractable oxidised N } (t_1) * \text{bulk density.}$$

2.3.7. Vegetation surveying

Cover estimates of vascular plants and mosses were made using 1m x 1m quadrats divided into 100, 10 cm x 10 cm divisions. Quadrats were placed adjacent to all 30 sampling locations at the three sites. The presence or absence of each species in each of the 100 sub-divisions was used to estimate plant cover at the 1 m² level.

Mean frequency weighted Ellenberg values were calculated for N, salt, pH and moisture using UK Ellenberg indicator values (Hill *et al.*, 1999). Species richness (S) was taken to be the total number of vascular plant species at each survey point.

Destructive harvests were carried out in August 2009 when many species were at maximum annual biomass. The use of both plant biomass and cover estimates for vegetation surveying reduced over-estimation of the abundance of functional groups which appeared frequently, but did not dominate cover. Vegetation from five randomly selected 10 cm x 10 cm sub-samples was cut to ground level and removed at all 30 sampling locations at the three sites. Samples were dried for 48 hours and plant litter (dead plant material not attached to living tissue) was removed since litter may have been transported away from its original location. Plant species were sorted into functional groups (grass, forb and moss) and weighed.

2.3.8. Statistics

To identify relationships between traffic volume, distance from the roadside and NO₂ concentrations, a restricted maximum-likelihood linear mixed effect model was used (LME; Pinheiro and Bates, 2001). In this model, transect nested within site was treated as a random effect with distance from the roadside and monthly traffic volume treated as fixed effects. The use of random effects in this context allows consideration of the nested nature of the dataset. Estimates of monthly traffic flows were calculated by multiplying annual traffic flows at the traffic count nearest each site with published proportions of journeys made in each month (DfT, 2011). Distance from the road was log transformed to provide linearity prior to statistical testing using R (version 2.11.1, www.r-project.org).

At each of the three sites, relationships between roadside proximity and the variables NO_2 , HM concentrations, moisture, pH, extractable soil N ($\text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$), mineralisation rates, nitrification rates, vegetation cover, species richness, plant biomass and frequency-weighted Ellenberg indicator values were identified using linear regression. Species richness relationships with distance from the road were identified using glm models with a Poisson error structure. Optimal patterns of relationship (linear, quadratic or logarithmic) were selected based on the highest r^2 values

Multiple regression analyses were then used to identify the key drivers of any relationships identified between biomass, plant cover, frequency-weighted Ellenberg indicator values and roadside proximity. This was achieved by assessing the role of the potential explanatory variables: measured NO_2 , modelled NH_3 , pH, moisture and extractable soil N. For each response variable an initial model was created, containing all fixed effects and interactions between fixed effects which could plausibly be used to explain variation in each response variable. Auto-correlated variables, identified by regression analyses, and optimal patterns of relationships (linear, quadratic or logarithmic) were introduced into models based on highest r^2 values. Where appropriate, model simplification of optimal initial models took place by using stepwise deletion to remove the highest order non-significant interaction terms, hence identifying significant terms (Crawley, 2007). Likelihood ratio deletion F-tests ensured the validity of model simplification.

2.4. Results

2.4.1. Atmospheric NO₂ and NH₃ concentrations

Mean annual concentrations nearest the roadside were $49 \mu\text{g m}^{-3} \pm 4$ (mean $\pm$ s.e), $30 \mu\text{g m}^{-3} \pm 5$ and $14 \mu\text{g m}^{-3} \pm 2$ for the high TS, medium TS and low TS respectively (figure 2.1). NO₂ concentration declined logarithmically with distance from the road at the high TS ($t = -8.0$, $p < 0.001$), medium TS ($t = -3.5$, $p < 0.001$) and low TS ($t = -1.5$, $p < 0.01$). Monthly mean roadside concentrations of NO₂ showed some variation, with maximum values of $73 \mu\text{g m}^{-3} \pm 4$ measured in July 2009 and minimum values of $28 \mu\text{g m}^{-3} \pm 3$ measured in September 2009 closest to the road at the high TS. Nearest the road at the medium TS, monthly mean concentrations of NO₂ ranged from $53 \mu\text{g m}^{-3}$ in February 2010 ± 4 to $19 \mu\text{g m}^{-3} \pm 3$ in December 2009, whereas at the low TS, concentrations ranged from $27 \mu\text{g m}^{-3} \pm 2$ in February 2010 to $9 \mu\text{g m}^{-3} \pm 1$ in May 2010.

Roadside mean NH₃ concentrations were modelled as $7.3 \mu\text{g m}^{-3}$, $7.1 \mu\text{g m}^{-3}$ and $4.3 \mu\text{g m}^{-3}$ at the high, medium and low TS, respectively. NH₃ concentrations above the critical level of $3 \mu\text{g m}^{-3}$ (AQEG, 2004) were estimated to persist up to 8 m within the high TS and throughout the remaining sites as background NH₃ concentrations (2009) were estimated (using 5 km x 5 km grid squares) to be greater than $3 \mu\text{g m}^{-3}$ (DEFRA, 2011). NH₃ input by vehicles are expected to decline by 99%, 20 m from the roadside at all three sites.

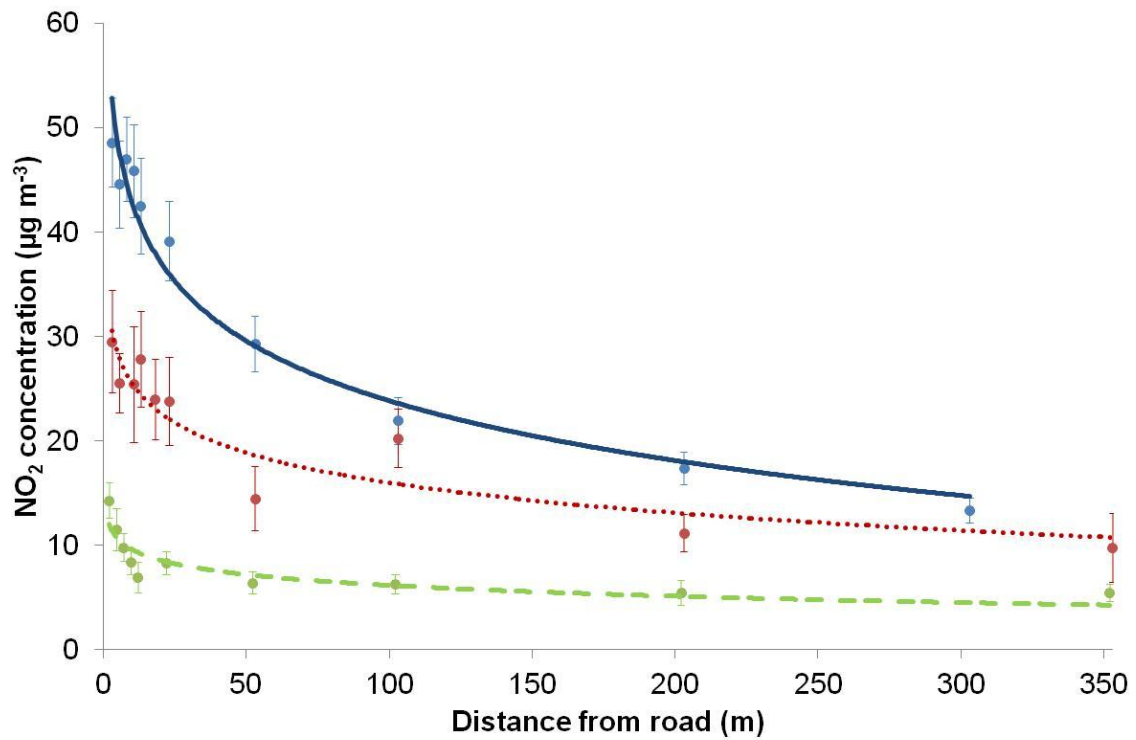


Figure 2.1: The negative logarithmic relationship between the mean concentrations of NO₂ (µg m⁻³) and distance from the road (m) at the high TS (blue continuous line), medium TS (red dotted line) and low TS (green dashed line). Values represent means ± s.e.

NO₂ concentrations were related to traffic volume and roadside proximity (Figure 2.2). There was a negative logarithmic relationship between NO₂ concentration and distance from the roadside ($t = -3.2$, $p < 0.001$), a positive linear relationship with increasing monthly traffic volume ($t = 12.9$, $p < 0.001$) and a significant interaction between distance and traffic volume ($t = -10.56$, $p < 0.001$).

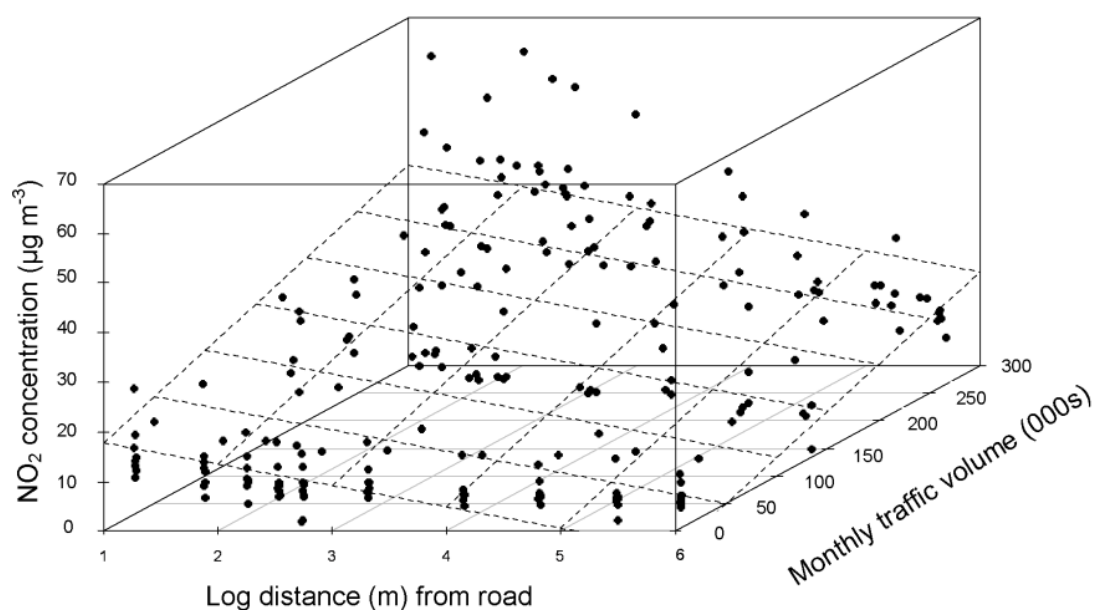


Figure 2.2: The relationship between the log transformed distance from the road (m) and the monthly traffic volume on monthly concentrations of NO_2 ($\mu\text{g m}^{-3}$) at all three sites. This plane represents a fitted relationship using linear regression, with each point representing the mean of 3 diffusion tubes exposed for a month. Random effects used in the model were not applied to the relationship shown here, which is for illustrative purposes only.

2.4.2. Heavy metals in the soil

Cu, Pb and Zn concentrations were approximately doubled in roadside soil relative to the concentrations found farthest from the road (Figure 2.3) and concentrations declined in a negative logarithmic relationship with increasing distance from the roadside. Nearest the road, mean concentrations of Zn, Pb and Cu across the three sites were $148 \text{ mg kg}^{-1} \pm 45$, $52 \text{ mg kg}^{-1} \pm 2$ and $12 \text{ mg kg}^{-1} \pm 2$ respectively. Zn declined the most rapidly, with mean concentrations declining to those measured >

300 m from the roadside within 4.5 m of the road edge. Approximately 43 % of the decline in mean Cu and Pb concentrations across transects occurred within 10 m of the first location marker. There was no evidence of a traffic volume effect on the concentration of any of the soil elements measured. No significant relationships were found between the remaining elements and roadside proximity (all $p > 0.05$).

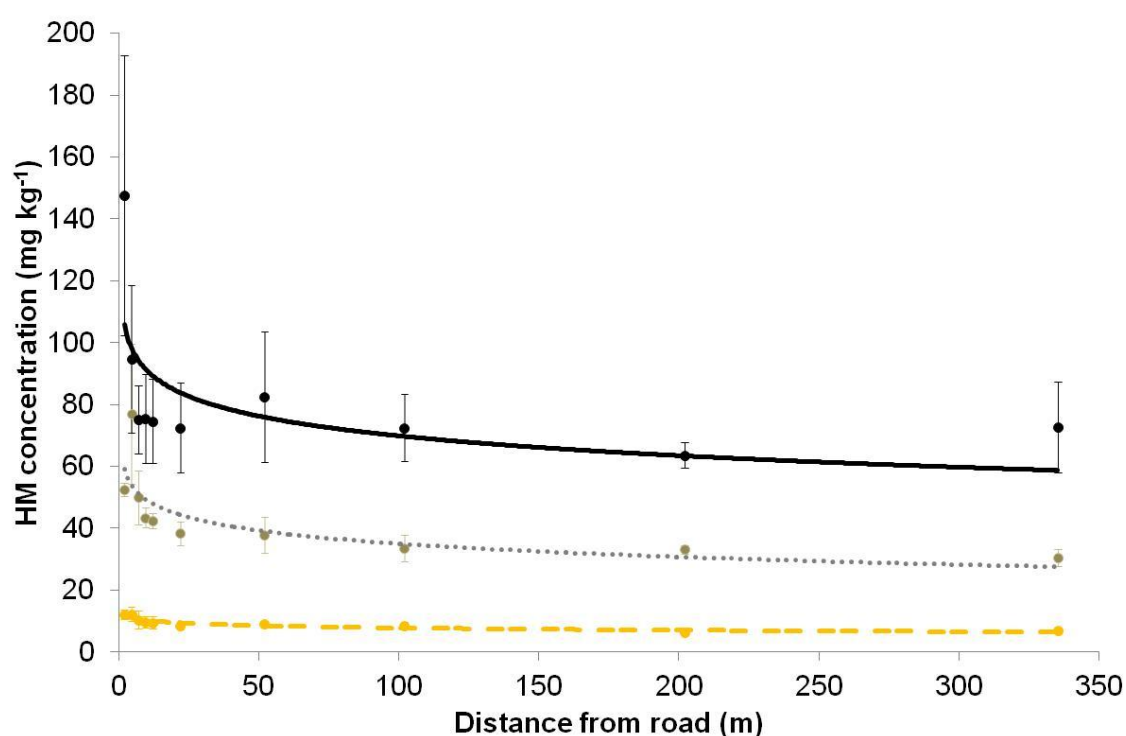


Figure 2.3: The negative logarithmic relationships between soil Zn (black continuous line), Pb (grey dotted line) and Cu (orange dashed line) concentrations and distance from the road (m). Values represent means $\pm$ s.e across all three sites

2.4.3. Soil moisture, pH and extractable N

The high TS had the wettest soil across all locations with mean soil moisture content of $29 \% \pm 2$, compared to $14 \% \pm 2$ and $12 \% \pm 2$ for the medium TS and low TS,

respectively. At the high TS, soil moisture declined with increasing distance from the road in March ($t = -3.9$, $p < 0.05$), May ($t = -2.7$, $p < 0.05$) and August 2010 ($t = -3.5$, $p < 0.01$). At the medium TS, soil moisture increased farther from the road in May ($t = -3.0$, $p < 0.01$) and, at the low TS, soil moisture declined away from the roadside in March 2010 ($t = -2.7$, $p < 0.05$) but no significant relationships were found at either site in the other survey months (all $p > 0.05$).

The relationships between pH and road proximity varied between sampling periods. In March 2010, pH was higher at the roadside and declined with distance from the road at all three sites (all $p < 0.05$, figure 2.4). The relationship between pH and distance from the road disappeared at the high TS and medium TS ($p > 0.05$) in May 2010 while the low TS retained a negative relationship with increasing distance from the road ($t = -6.6$, $p < 0.001$) at this time. In August 2010, there were no relationships between roadside proximity and pH at any of the three sites (all $p > 0.05$).

Extractable N was not significantly related to roadside proximity at either the medium TS or low TS, but increased with increasing distance from the roadside at the high TS ($t = -1.3$, $p < 0.01$). Annual mineralisation and nitrification rates were not related to roadside proximity at any of the three sites (all $p > 0.05$). In winter, mineralisation ($t = -3.4$, $p < 0.01$, appendix 2.1) and nitrification rates declined ($t = -2.1$, $p < 0.05$) away from the roadside at the high TS. In the summer, mineralisation rates ($t = -3.4$, $p < 0.01$) and nitrification rates ($t = -3.1$, $p < 0.01$) also declined away from the road at the medium TS.

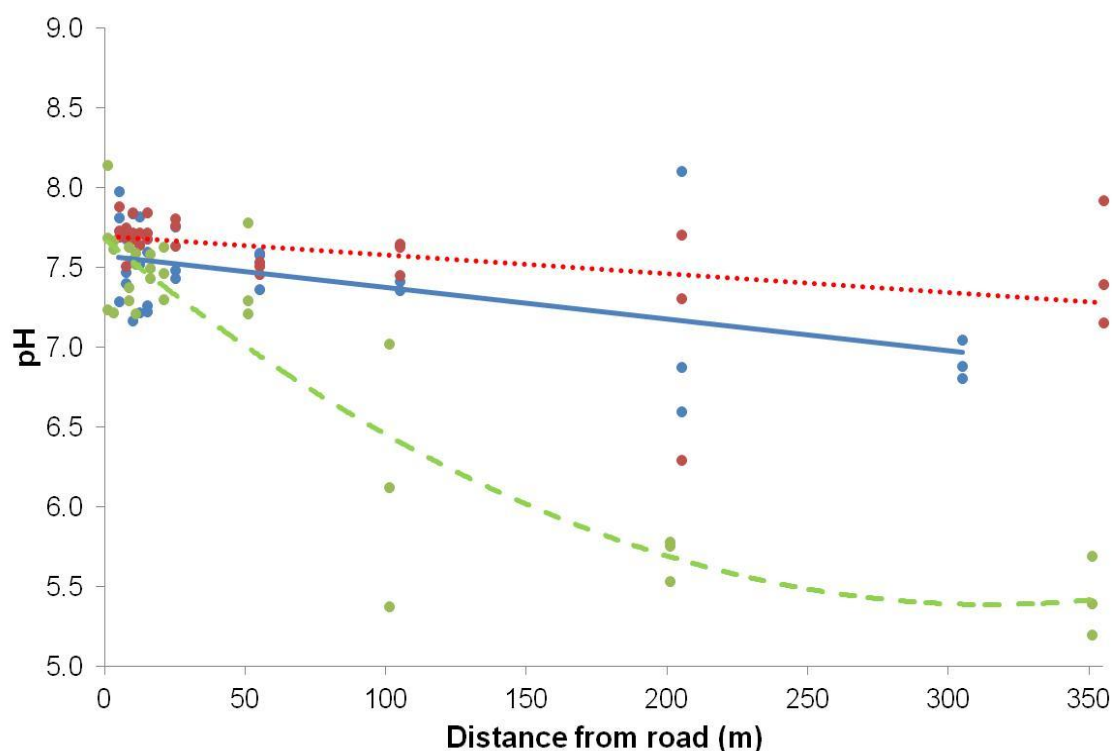


Figure 2.4: The relationship between the pH and distance from the road in March 2010 at the high TS (blue continuous line), medium TS (red dotted line) and low TS (green dashed line).

2.4.4. Plant composition and productivity

49, 48 and 49 plant species were identified at the high TS, medium TS and low TS respectively (for a full list of species present at each site see appendix 2.2). Sites were similar overall with respect to the species present and 17 species were common to all sites. Total plant species richness declined nearer the roadside at the high TS and the low TS, with a mean of 11 species m^{-2} and 8 species m^{-2} found at the roadside at each site, respectively (table 2.2). Further from the road, species richness increased to a mean of 14 species m^{-2} , 350 m from the road edge at both sites.

Table 2.2: A summary of regression analyses showing relationships between the stated variable and distance from the roadside. + denotes that the variable increases with increasing distance from the roadside, – denotes a decline with increasing distance from the roadside and ns denotes non-significance at $p > 0.05$. Linear, logarithmic (log) and quadratic (quad) denote the shape of the relationship ($n = 29$).

Variable	High trafficked site	Medium trafficked site	Low trafficked site
Grass biomass	- linear ($t = -3.8$, $p < 0.001$)	ns	ns
Forb biomass	ns	ns	ns
Moss biomass	ns	+ quad ($t = 2.1$, $p < 0.05$)	+ linear ($t = 3.1$, $p < 0.01$)
Total biomass	- quad ($t = -2.9$, $p < 0.01$)	ns	ns
Grass cover	ns	ns	ns
Forb cover	+ log ($t = 3.6$, $p < 0.01$)	ns	+ log ($t = 2.7$, $p < 0.05$)
Moss cover	- quad ($t = -3.1$, $p < 0.01$)	ns	+ log ($t = 3.8$, $p < 0.001$)
Species richness	+ log ($z = 4.9$, $p < 0.001$)	ns	+ log ($z = 2.7$, $p < 0.01$)
Ellenberg N	ns	- log ($t = -4.1$, $p < 0.001$)	- log ($t = -3.1$, $p < 0.01$)
Ellenberg pH	+ log ($t = 2.3$, $p < 0.05$)	- log ($t = -4.7$, $p < 0.001$)	- log ($t = -2.1$, $p < 0.05$)
Ellenberg moisture	+ log ($t = 2.8$, $p < 0.01$)	ns	ns
Ellenberg salt	ns	ns	ns

Grass biomass was generally higher than mosses or forbs, with a mean dry mass of $266 \text{ g m}^{-2} \pm 20$ (mean $\pm$ s.e) across all sites and transects. Mean biomass of mosses and forbs were $28 \text{ g m}^{-2} \pm 8$ and $20 \text{ g m}^{-2} \pm 3$ respectively. Total plant biomass and grass biomass declined with distance from the road at the high TS, whereas moss biomass increased further from the road at the medium TS and low TS. Forb biomass was not related to roadside proximity at any of the three sites but forb cover and overall plant species richness increased away from the road at the high TS and low TS.

Frequency-weighted Ellenberg indicator scores for N and pH declined away from the road at the medium TS and low TS, but there was no significant relationship with roadside proximity at the high TS. There was also a significant positive relationship between NO₂ concentration and Ellenberg N scores at the medium TS ($t = 2.7$, $p < 0.05$) and the low TS ($t = 3.9$, $p < 0.01$). However, there was no relationship between soil N, NH₃, pH or moisture and their respective Ellenberg scores at any of these sites (all $p > 0.05$). At the high TS, Ellenberg scores for pH and moisture significantly increased with increasing distance from the road, but Ellenberg N scores were not positively correlated with measured NO₂, NH₃, soil N, pH or moisture (all $p > 0.05$). Ellenberg salt scores were not related to roadside proximity at any of the sites ($p > 0.05$).

Total plant biomass and grass biomass were both positively related to NO₂ concentrations at the high TS (table 2.3). At the medium TS total biomass was related to increased extractable soil N but none of the measured variables adequately explained the variation in total biomass at the low trafficked site. Forb cover declined with increasing soil moisture at the high TS and with increasing NO₂ concentration at the medium TS. Moss biomass was lower where NO₂ levels were higher at the low TS but was not associated with any of the measured drivers of change at the high TS and medium TS.

Table 2.3: A summary of the minimal adequate model from the multiple regression analyses for each site. Terms in initial multiple regression model: measured mean NO₂, modelled mean NH₃, and: moisture, pH, extractable soil N (ext soil N) and their interactions (n = 29). Optimal initial models were selected based on the model with the highest r² values. Relationships given are linear. Logarithmic and quadratic relationships were also tested but explained less of the variation in the data.

Variable	Site	MaM	Value	df	t	p	r ²
Total biomass (g)	High TS	Intercept NO ₂	20.3 1.7	28	2.5	< 0.05	0.15
	Medium TS	Intercept Ext soil N	14.2 1.7	25	2.8	< 0.01	0.21
	Low TS	n/a				ns	
Grass biomass (g)	High TS	Intercept NO ₂	3.5 1.7	28	3.9	< 0.001	0.33
	Medium TS	n/a				ns	
	Low TS	n/a				ns	
Forb cover (%)	High TS	Intercept Moisture	249.0 -4.1	28	-4.9	< 0.001	0.44
	Medium TS	n/a				ns	
	Low TS	Intercept NO ₂	135.9 -8.2	28	-2.1	< 0.05	0.17
Moss biomass (g)	High TS	n/a				ns	
	Medium TS	n/a				ns	
	Low TS	Intercept NO ₂	16.1 -1.4	27	-2.4	< 0.05	0.14

2.5. Discussion

2.5.1. The influence of roads on air quality

Mean annual concentrations of NO₂ were significantly elevated at the roadside, compared to background locations (> 300m away) at all three sites, indicating that vehicles were the primary source of this pollutant. Substantial areas of the high TS and medium TS were exposed to mean annual concentrations above the critical level of NO_x for the protection of vegetation and ecosystems of 30 µg m⁻³ (AQEG, 2004). Between 3.2% and 23.5% of NO_x emitted by motor vehicles is emitted as NO₂ (Carslaw & Beevers, 2005), hence NO_x (NO + NO₂) concentrations at the roadside are likely to be considerably higher than the NO₂ concentrations reported here.

The three dimensional relationship presented in this study indicates that traffic volume and distance from the roadside can be used together to predict NO₂ concentrations alongside roads, where background concentrations are approximately constant (Carslaw *et al.*, 2011). Monthly mean NO₂ concentrations may be estimated using monthly traffic volumes and distance from the roadside using formula 4, where D = distance from the roadside (m) and T = monthly traffic volume. Published NO₂ exponential decay rates vary widely from 0.005 m⁻¹ to 0.15 m⁻¹ depending upon several factors including the maximum distance that NO₂ was measured and the prevailing wind direction (for a summary see Cape *et al.*, 2004). Fitting an exponential function to the data from the three calcareous sites yielded slow decay rates of 0.004 m⁻¹, 0.003 m⁻¹ and 0.002 m⁻¹ at the high TS, medium TS and low TS, respectively. However, formula 4 uses logarithmic functions because declines in NO₂ concentrations away from roadsides are more accurately described.

$$(4) \text{ NO}_2 \text{ concentration } (\mu\text{g m}^{-3}) = 11.60 - 1.23 \ln D + 0.000014 T - 0.000002 \ln DT$$

The higher deposition velocity of NH_3 compared with NO_2 means that vehicular derived NH_3 is unlikely to extend over as large an area as NO_2 . This is consistent with a 90% decline in measured NH_3 concentrations within 10 m of roadsides across a range of traffic densities in Scotland (Cape *et al.*, 2004). Variation in emissions ratios of NH_3 compared with NO_2 from heavy good vehicles (HGVs) were not considered in this study as the ratio of HGV to other vehicles was consistently low; 0.09 ± 0.001 , 0.05 ± 0.002 and 0.05 ± 0.002 at the high TS, medium TS and low TS, respectively (DfT, 2011).

Viskari *et al.* (1997) estimated that background levels for PAH emissions were reached at a distance of between 60 and 100 m from the roadside. The dispersal characteristics of other exhaust emissions (CO_2 , CO, CH_4 , HNO_2 and VOCs) are less well understood, but are also likely to be elevated at roadsides. Despite meta-analyses linking CO_2 enrichment with increasing plant productivity across fumigation experiments (Lee *et al.*, 2010), the effects on roadside vegetation of the other unmeasured exhaust gas fractions (including CO_2) are poorly understood and warrant further investigation.

2.5.2. Road induced changes to the soil physical and chemical environment

Soils were wettest next to the road at the high TS, becoming drier with increasing distance away from the roadside. Most modern roads are impermeable to water and are curved in design to allow efficient drainage (DfT, 2011). The pattern of increasing soil moisture at the high TS seems likely to be related, at least in part, to

the substantial area of tarmac which may result in substantial disruption to natural drainage.

Run-off from roads not only changes the hydrology of soils, but has also been shown to transport HMs and de-icing salts away from the road (Backstrom *et al.*, 2003). Zn, Pb and Cu declined logarithmically with distance from the roadside at all three study sites but concentrations were unrelated to traffic density. Cu and Zn have been shown to be derived from petrol and diesel exhaust emissions, lubricating oil, brake wear and tyre erosion (for a summary see Zechmeister *et al.*, 2005). Elevated Pb at the roadside may represent a legacy of the use of leaded petrol, deposition from brake wear or diesel exhaust emissions (Laschober *et al.*, 2004, Wang *et al.*, 2007a). Mean Zn concentrations(+ 9 %) were moderately higher than concentrations measured alongside a UK motorway over twenty years ago, when traffic densities will have been approximately two-thirds of current densities (DfT, 2011), however Pb (- 36 %) and Cu (-60 %) concentrations were considerably lower in our study (Warren & Birch, 1987).

N mineralisation rates increased nearer the road for one quarter at the high TS (winter) and at the medium TS (summer), however, annual mean extractable soil N declined nearer the road at the high TS with no relationships being found at the remaining two sites. In addition studies on calcareous grasslands have demonstrated that up to 89% of N can be retained in calcareous soils (Phoenix *et al.*, 2003). In roadside environments, N inputs from road vehicles may be immobilised by microbial uptake (Bardgett *et al.*, 2003), leached out of soils (Jaber *et al.*, 2005), used to increase plant productivity (Lee *et al.*, 2010) and/or result in increased plant tissue N concentrations (Laffray *et al.*, 2010). Increased denitification rates nearer

the roadside will also reduce soil N if run-off from road surfaces results in waterlogged soil (Wilson *et al.*, 1995).

2.5.3. Road induced changes to adjacent vegetation

Ellenberg N indicator scores increased at the roadside at the medium TS and low TS. Ellenberg N scores were positively correlated with NO₂ gradients at both sites but were not related to estimated NH₃ concentrations at any of the sites, suggesting that elevated NO₂ deposition may be driving the increased abundance of N-tolerant species. NH₃ has been associated with plant species changes in experimental ecosystems (e.g. Sheppard *et al.*, 2011), however the anticipated rapid decline in NH₃ concentrations close to the roadside means plant compositional changes across gradients of N enrichment at the medium TS and low TS are more likely to be driven by NO₂. A lack of relationship between Ellenberg N and roadside proximity at the high TS may be due to the influence of soil moisture which was associated with declining forb abundance at that site.

Higher total plant biomass accumulation nearer the roadside was also correlated with NO₂ gradients at the high TS. This relationship was driven primarily by grasses which comprised 85% of the total biomass at this site. Meta-analyses of N enrichment studies have shown increases in grassland productivity in response to N (LeBauer & Treseder, 2008) but local conditions, such as phosphorus limitation, climate and moisture regime, are likely to dictate whether N enrichment increases productivity in calcareous grasslands (Wilson *et al.*, 1995, Carroll *et al.*, 2003). In addition to the direct effects of N enrichment, roads may also influence roadside plant communities indirectly through other environmental changes, for example via preferential grazing and/or increases in plant tissue HM concentrations including Cu and Zn which are essential to plant growth and development (Pilon *et al.*, 2006).

The rapid decline in HM concentrations within a few metres of the road and the low measured concentrations of Cu and Pb means that elevated HM concentrations are unlikely to account for a large amount of the variation in plant community composition across the transects.

Forb biomass was not related to roadside proximity, although forb cover increased away from the road at the high TS and the low TS. The lack of a biomass response is probably because large changes in forb % cover resulted in only small changes in overall biomass. Increasing forb cover was associated with decreased NO₂ concentrations at the low TS and decreased soil moisture at the high TS.

Calcareous forb species are generally slow growing and are associated with low nutrient availability and high drought tolerance (Critchley *et al.*, 2002, Bennie *et al.*, 2006). Nutrient enrichment and changes to drainage brought about by roads may, therefore, result in a shift in dominance from forbs to grasses nearer the roadside with an associated decline in plant species richness.

The N tolerant moss, *Brachythecium rutabulum* (Pitcairn *et al.*, 2003), was present at the high TS and medium TS, whereas the intermediate N moss species *Pseudoscleropodium purum* was present at the medium and low TS (Bates, 1994) and N sensitive *Amblystegium serpens* (Krommer *et al.*, 2007) was found only at the low TS. At low concentrations ($\sim 1 \mu\text{g m}^{-3}$), NH₃ has been demonstrated to increase moss tissue N content and may be playing a role in the calcareous moss community (Pitcairn *et al.*, 2006). However, since none of the measured or modelled environmental drivers were related to moss biomass at the medium TS or high TS, it is more likely that the variation in moss abundance was due to competitive exclusion by other plant functional groups rather than the direct effects of exhaust emissions (Virtanen *et al.*, 2000).

Roadside plant species composition may also be influenced by the trees or shrubs used to border roads. Across the three sites, the shrubs *Cretagus monogyna* and *Berberis vulgaris* were present at the road edge. Trees and shrubs can provide barriers which limit the dispersal of exhaust emissions (Yin *et al.*, 2011) and are frequently used for this purpose. Although salt tolerant species have also been found to be more abundant at the roadside in another study (Scott & Davison, 1985), salt dispersal has been shown to be limited, declining by 70%, ~2 m from the road edge (Thompson *et al.*, 1986). Exotic species can also be distributed in tyre tread (Gelbard & Belnap, 2003, Sera, 2010) and likely future changes to climate may make conditions more favourable to invasion.

2.6. Conclusions

We present evidence that roads can bring about a suite of environmental changes, including changes in atmospheric pollution, soil biogeochemistry and hydrology. Measured increases in the abundance of N tolerant plant species, and an associated decline in species richness at the roadside in two calcareous ecosystems adds to the growing evidence from several ecosystem types that N enrichment is a key driver of change in roadside ecosystems. Roadside environmental conditions also favoured grasses over forbs resulting in increases in community productivity. The findings from this study further our understanding of many of the threats from roads and road vehicles on plant community composition and function in adjacent ecosystems of high conservation value.

3. Direct and indirect effects of roads and road vehicles on the plant community composition of calcareous grasslands

3.1. Abstract

Exposure of roadside plants to vehicle exhaust emissions and road-induced changes to soil biogeochemistry have been cited as possible mechanisms for shifts in plant communities nearer roadsides in calcareous grasslands. A suite of environmental variables were measured along transects away from roads of varying traffic flow rates across eight calcareous grasslands. Dry ammonia (NH_3) and dry nitrogen dioxide (NO_2) deposition gradients away from the roads were estimated based on a model constructed from measurements taken at three of the eight sites. Total (dry + wet) nitrogen (N) deposition rates were also obtained. This multi-site approach allowed mixed effects modelling to identify the likely drivers of observed plant compositional changes. Increases in abundances of nitrophilic plant species at roadsides were associated with increased NO_2 deposition, but not with NH_3 or total N deposition. Forb abundance and diversity increases at roadsides were also associated with NO_2 deposition, with relationships driven by increases in typical edge plant species and not species characteristic of calcareous grasslands. Current management practises (grazing livestock and mowing) will not prevent deposition of vehicle exhaust products to plants and soils or prevent changes to soil biogeochemistry driven by road run-off. With calcareous grasslands declining,

quantification of the extent of road influences on plant communities may inform management decisions aimed at protecting these diverse landscapes.

3.2. Introduction

Calcareous grasslands are among the most species rich ecosystems in Western Europe and are important habitats for a diverse flora and fauna (Mortimer *et al.*, 1998). Calcareous grassland and roadside verges supporting calcareous plant communities are priority habitats for the protection of biodiversity in the UK (UKBAP, 2007) and across Europe (Pedro Silva *et al.*, 2008). Whilst species rich grasslands are important from a conservation perspective they are reducing in area. Calcareous grasslands have declined by 18,000 ha in the UK (1984 – 2007) and, in total, natural grasslands have declined by 180,000 ha across Europe (1990 – 2006) as the result of road building, pollution, urbanisation, afforestation and conversion to agricultural land (Countryside-Survey, 2008, EEA, 2010).

Roads are currently estimated to extend 400,000 kilometres in the UK (DfT, 2011) and 5 million kilometres across Europe (ERF, 2010). Plants growing alongside these roads can be directly exposed to elevated concentrations of several pollutants emitted from vehicle exhausts and engine degradation, including nitric oxide (NO), nitrogen dioxide (NO₂), ammonia (NH₃), heavy metals (HM), poly-aromatic hydrocarbons (PAH), volatile organic compounds (VOCs) and carbon dioxide (CO₂) (Wallington *et al.*, 2008). Plants can also be influenced by roads indirectly via run-off; increasing soil moisture content (Boivin *et al.*, 2008), transporting alkaline road surface chemicals which raise soil pH (DfT, 2008), depositing suspended HMs and depositing dissolved salt to roadside soils (Zechmeister *et al.*, 2005, Li *et al.*, 2007). Rapidly moving traffic can also increase the frequency of roadside disturbances e.g. wind turbulence (Truscott *et al.*, 2005).

Directs effects of road vehicles on plant phenology including changes to growth rates, flower development and rates of leaf senescence can be demonstrated by controlled fumigations with exhaust gases. Fumigation studies frequently attribute variation in plant responses to the extent different species assimilate or detoxify additional gaseous N (e.g. Honour *et al.*, 2009, Viskari *et al.*, 2000). Similarly, changes to plant species composition alongside roads have been correlated with local gradients of N enrichment; in heathland (Angold, 1997), in the ground flora of coniferous forests (Bernhardt-Romermann *et al.*, 2007), at a blanket bog (Bignal *et al.*, 2007) and within roadside verge plant communities (Truscott *et al.*, 2005). Roadside studies are complemented by observed declines in plant species richness in acidic grasslands, and reduced Shannon diversity (and evenness) indices in calcareous grasslands across national gradients of N deposition (Stevens *et al.*, 2004, Van den Berg *et al.*, 2011).

Calcareous grasslands are particularly sensitive to the suite of environmental changes associated with roads because associated plant species are typically adapted to a relatively narrow range of ecological conditions, particularly alkaline soils, exposure to frequent drought events and low nutrient availability (Critchley *et al.*, 2002, Bennie *et al.*, 2006). Critical levels (CLE), above which ecological changes are anticipated, for NO_x and NH₃ are annual mean concentrations of not more than 30 µg m⁻³ and 3 µg m⁻³, respectively (WHO, 2000, DEFRA, 2007). CLEs are constant across all ecosystems, including sensitive ecosystems such as calcareous grasslands. Critical loads (CLOs) vary according to ecosystem type, for example the CLO for total N deposition in calcareous grasslands is between 15 and 25 kg N ha⁻¹ yr⁻¹ (Bobbink & Hattelingh, 2011). Significant changes in the growth rates of

grassland plants at relatively low N inputs, however, suggest that current CLOs and CLEs may be too high for some sensitive grasslands (Bobbink *et al.*, 2010).

Land managers commonly use grazing livestock (sheep and/or cattle) or mowing to prevent the accumulation of soil nutrients, to remove small populations of invading plant species and to inhibit successional changes in semi-natural calcareous habitats (Kahmen *et al.*, 2002). Infrequently used management regimes alongside roads includes physical barriers (e.g. trees) which can impede the dispersal of vehicle exhaust derived pollution, buffer zones to intercept road run-off and/or active filters which remove pollutants in transit (for a review see Spellerberg, 1998). Quantifying the region of influence of roads within calcareous grassland ecosystems is important if land managers are to select the most appropriate management regime to protect the structure and function of these species-rich ecosystems.

Roadside plant communities provide a unique opportunity to assess the effects of long-term exposure to gradients of environmental change, including gradients of N enrichment. Roadside N enrichment and elevated soil moisture content has been associated with localised increases in the abundance of grasses and reductions in forbs as well as an increase in N and moisture tolerant species in three calcareous grasslands (Lee *et al.*, 2012). The detailed study by Lee *et al.*, (2012) measured a range of parameters informing the methodology of the multi-site investigation presented in this article. Measurements of hydrology, biogeochemistry and plant species composition at eight calcareous grassland sites, coupled with modelled N deposition rates, allowed a mixed effects modelling approach to: (1) test the ubiquity of the relationships observed across the three site study, (2) identify which plant species and groups of plant species are increasing and decreasing at roadsides, (3) establish the likely environmental drivers of observed relationships and (4) provide

quantitative data which can inform management decisions aimed at reducing the influence of roads on nearby calcareous grasslands.

3.3. Materials and methods

3.3.1. Sites

Eight grass-dominated ecosystems growing on chalk substrate were selected to represent species rich calcareous grassland communities with high conservation value (Rodwell, 1992) at sites with a range of slopes and aspects (for site details see table 3.1). Sites were; Aston Rowant (AR), Butser Hill (BH), Lewes Down (LD), Newlands Corner (NC), Martins Down (MD), Seale Chalk Pit (SCP), St Catherine's Hill (SCP) and Stockbridge Down (SBD) located across Southern England. Three parallel transects were set up approximately 50 – 75 m apart at each site, perpendicular to the road. Transects began as near to the road as possible within the boundaries of each site and then continued a further 2.5, 5, 7.5, 10, 20, 50, and 100 m. There were, therefore, 24 location markers at each site and a total of 192 survey locations. Study sites were located in close proximity to roads (mean distance from road to site edge = 6 m) with a range of traffic flow rates. Management at each site used grazing sheep or cattle and/or cutting with a view to conserve the calcareous grassland community.

Table 3.1: Details of study sites including; annual traffic volume (million vehicles, T), management type (grazing, G and/or cutting, C), background nitrogen (N) deposition, background NO₂ deposition (DEFRA, 2011), elevation of the first location marker above (+) or below (-) the road, slope away from the road (incline, ↑, decline, ↓ or none, →), location and rainfall. Conservation designations are: National Nature Reserve (NNR), Site of Special Scientific Interest (SSSI) and Area of Outstanding Natural Beauty (AONB). Mean temperatures range from 9 °C to 10 °C with annual rainfall given (MetOffice, 2011). Grazing rates are given as the number of sheep per hectare (cattle per hectare at NC).

Site	T*	Management (grazing density)	N (NO ₂) dep Kg N ha ⁻¹ yr ⁻¹	Elevation (m)	Slope	Location N E		Rainfall (mm yr ⁻¹)
Aston Rowant NNR	35.0	G ^a (2.5 – 2.7)	10.9 (0.5)	0	↑	51.688	-0.949	500 - 600
Butser Hill NNR	17.1	G ^b (1.1 – 2.1)	13.6 (0.4)	10	↑	50.979	-0.975	800 - 1000
Lewes Down NNR	8.3	na ^c	11.4 (0.3)	0	↑	50.876	0.021	800 - 1000
Martins Down NNR	2.4	G ^d (0.9 – 1.9)	14.6 (0.6)	0	→	50.968	-1.536	600 - 800
Newlands Corner AONB	5.3	G ^e (1.0 – 1.2)	10.3 (0.4)	-2	↓	51.232	-0.506	600 - 800
St Catherine's Hill SSSI	38.2	G ^f (3.4 – 3.7)	15.5 (0.7)	38	↓	51.044	-1.310	600 - 800
Seale Chalk Pit SSSI	17.9	C ^g (n/a)	9.2 (0.4)	0	↓	51.224	-0.714	600 - 800
Stockbridge Down AONB	3.2	GC ^h (1.0 – 1.5)	11.6 (0.6)	0	→	51.098	-1.409	600 - 800

* DfT, 2011, ^a R.Silverwood, *pers comm*, ^b T.Speller, *pers comm*, ^c S.Tillman, *pers comm*, ^d L.Smith, *pers comm*, ^e C.Williams, *pers comm*, ^f A.Watson, *pers comm*, ^g M.Allen, *pers comm*. and ^h estimated from observations on-site.

3.3.2. Soil HM concentrations

Soil samples were collected at each of the 192 survey locations using 2.5 cm diameter, 15 cm length polyvinyl chloride (PVC) tubes in March 2011. Samples were homogenised and dried in a drying oven ($80\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$) for 48 hours. 1 g ($\pm 0.01\text{ g}$) sub samples were refluxed with 1:1 65% nitric acid and deionised water, 30% hydrogen peroxide and hydrochloric acid at $95\text{ }^{\circ}\text{C}$ (following the methods outlined in van der Wiel, 2003). HM content was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, Agilent Technologies, UK) by comparison with standard reference material (SRM 2704, Buffalo river sediment). Metals measured were lead (Pb), copper (Cu), zinc (Zn), vanadium (V), nickel (Ni), chromium (Cr), cobalt (Co), cadmium (Cd) and arsenic (As).

3.3.3. Soil pH, salinity and moisture content

Soil pH was measured at each site in June 2011 using a PHH-200 pH probe (Omega, UK). Soil moisture was measured using a ThetaProbe soil moisture sensor (Delta-T, UK) in April 2011. Soil moisture and pH were measured at 5 cm soil depths with three replicates at each of the 192 sampling locations. The timing of soil moisture and pH surveys was directed by the study of Lee *et al.*, (2012), as the most likely time to observe relationships between these variables and roadside proximity. For salinity measurements, soil samples were collected in February 2011, from each site and oven dried at $80\text{ }^{\circ}\text{C}$ for 48 hours. Soil was mixed with water in a 1:1 ratio (1 g soil: 1 ml water), shaken for 30 minutes and salinity was measured using a PHR-986 sodium-selective electrode (ThermoScientific, UK) by comparison with prepared standards (Zhang *et al.*, 2005). Re-analysis of 10% sub-samples ensured the accuracy of analytical methods.

3.3.4. Modelling NO₂ concentrations and N deposition

Studies have frequently fitted exponential decay functions to describe NO₂ declines away from roadsides, but published decay rates can vary widely (0.005 m⁻¹ to 0.15 m⁻¹) depending, in part, on the distances away from the road that measurements were taken (for a review see Cape et al., 2004). Across the eight calcareous grasslands used in this study, there was variation (1 – 32 m) in the distances between the roads and sites themselves. NO₂ declines away from roadsides were, therefore, modelled at each site using formula 1 (D = distance from the roadside and T = mean monthly traffic volume) which was derived from a year-long study at three of the grassland sites, AR, BH and MD. Monthly traffic densities were acquired from the nearest traffic count site (DfT, 2011).

NH₃ concentrations were modelled assuming a ratio of NO₂:NH₃ of 5:1 of the vehicular input NO₂ (i.e. above background concentrations) at the roadside (Cape et al., 2004), and were modelled to decay according to formula 2, where C_{bkgd} is the background concentration of NH₃ and C_{veh} is the estimated concentration of NH₃ nearest the road. Dry NO₂ and NH₃ deposition (F) were estimated from gaseous concentrations using formula 3, where V_d is the deposition velocity and C is the annual average concentrations. V_d for NO₂ and NH₃ were 0.003 and 0.015, respectively (Hanson & Lindberg, 1991, Harrison et al., 1996). Wet deposition rates (NH₄⁺ and NO₃⁻) and background concentrations of NH₃ and NO₂ for each site were obtained from 5 km² resolution deposition maps (DEFRA, 2011).

$$(1) \text{ NO}_2 = 11.6 - 1.23 \ln D + 0.000014 T - 0.000002 \ln DT$$

$$(2) \text{ NH}_3 = C_{\text{bkgd}} + C_{\text{veh}} e^{-0.25D}$$

$$(3) F = V_d \times C$$

3.3.5. Model testing

To test modelled NO₂ concentrations against measured NO₂ concentrations, Palmes diffusion tubes (7.1 cm long, diameter 1.1 cm) were deployed in triplicate at 10 distance markers, nearest the road and then a further 2.5 m, 5 m, 7.5 m, 10 m, 20 m, 50 m and 100 m, along one transect at SCP, SCH and NC. The absorbent medium contained within each tube was 2,2',2''-nitritoltriethanol (C₂H₄O₃N), which has a high capacity to remove NO₂ from the sampled air (for further details on tube preparation see Palmes *et al.*, 1976, Cape., 2009). The three sites were selected to provide a range of topographical features which were not considered in earlier NO₂ model construction (measured NO₂ concentrations at AR, BH and MD were used to construct the model). The grassland at SCH was 38 m above the road, at SCP there were physical barriers between the site and the road in the form of a belt of deciduous trees (~6 m thick) and at NC the grassland sloped down below the elevation of the road. The monitoring period was for two months, October and November 2010.

3.3.6. Vegetation surveying

Cover estimates of vascular plant were made using 1m x 1m quadrats divided into 100, 10 cm x 10 cm sub-quadrats. Quadrats were randomly placed adjacent to all sampling locations at all eight sites. The presence or absence of each species in each of the 100 sub-quadrats was used to estimate coverage at the 1 m² level. Average frequency-weighted Ellenberg values were calculated for N, salt, reaction (pH) and moisture using UK Ellenberg indicator values (Hill *et al.*, 1999). Species richness (S) was given as the total number of vascular plants (a full list of species identified at each site is given in appendix 3.1).

Nitrophilic, pH tolerant (at least moderately acid or alkali tolerant) and hydrophilic species were classified as species with Ellenberg scores greater than or equal to five. Salt tolerant species had Ellenberg scores greater than or equal to 1. Species which have been experimentally demonstrated to be tolerant to elevated HM concentrations were considered metallophytic. These species were *Arrhenatherum elatius* (Deram *et al.*, 2008), *Festuca ovina* (Brown & Brinkmann, 1992), *Rumex acetosa* (Barrutia *et al.*, 2009), *Plantago lanceolata* and *Silene vulgaris* (Baroni *et al.*, 2000). Typical calcareous plant species, plant species which are commonly found at the edge of habitats (edge species) and invasive species were assigned using the PLANTATT database (Hill *et al.*, 2004).

3.3.7. Statistical Analysis

Restricted maximum-likelihood linear mixed effect models (LME) were carried out using R (www.r-project.org, version 2.11.1) to identify relationships between HM concentrations, pH, moisture, frequency-weighted Ellenberg indicator scores and the abundance of individual species and groups of species, with traffic volume and roadside proximity (Pinhiero & Bates, 2001). Site was treated as a random effect and distance from the roadside and traffic volume were treated as fixed effects in LME. Allocating site as a random effect within LME ensured that natural variation between sites was accounted for and spatial pseudo-replication was avoided. The use of random effects in this context allows consideration of the nested nature of the dataset, with transects nested within sites. Relationships between roadside proximity and total species richness, grass species richness and forb species richness were identified using generalised linear models (GLM) with Poisson error structures.

LME analyses were also used to identify relationships between frequency-weighted Ellenberg scores (N, pH, salt and moisture) and their respective measured or modelled equivalents. The key drivers of changes within groups of plant species were identified using the sum of the abundances of the plant species classified as typical calcareous species and edge species, as well as cover estimates of the different functional groups, against the potential explanatory variables; modelled N deposition (dry NO₂, dry NH₃, wet N and total N), soil moisture and pH using LME. Site was treated as a random effect and N deposition, moisture and pH were treated as fixed effects in LME. Co-correlated variables were tested using linear regression and, where appropriate, selection between co-correlated variables was based on the lowest AIC value.

For each response variable that used LME, an initial model was created, containing all fixed effects and interactions between fixed effects which could plausibly explain the dataset. Model simplification of initial models took place using stepwise deletion to remove the highest order non-significant interaction term, thus identifying significant terms. For all statistical tests, selection of the optimal pattern of the relationship (linear, logarithmic or quadratic) was identified by sequentially introducing transformed and untransformed terms into initial models and selecting models with the lowest AIC (Crawley, 2007).

3.4. Results

3.4.1. Modelled gaseous N and N deposition

Modelled annual mean NO_2 concentrations ranged from $13 \mu\text{g m}^{-3}$ (MD) to $45 \mu\text{g m}^{-3}$ (AR) at roadside locations across the eight sites. NH_3 concentrations were estimated to range from $7.3 \mu\text{g m}^{-3}$ (AR) to $2.3 \mu\text{g m}^{-3}$ (SCH) nearest roadsides. As modelled gaseous concentrations of NH_3 and NO_2 declined logarithmically from the road edge, the estimated contribution of dry N deposition to total (wet + dry) N deposition declined from a mean across all sites of 76 % nearest the road to a mean of 63 %, ~ 100 m from the road. The ratio of oxidised to reduced N was also expected to vary away from the road, since NH_3 concentrations are modelled to decline more rapidly and closer to the road edge than NO_2 . As a result, the contribution of dry deposition of NH_3 to total N deposition rates was modelled to be negligibly above those which would be expected from background concentrations ~ 20 m from roadsides. Modelled total N and dry NO_2 deposition rates were estimated to exceed background deposition rates across the full transect at all sites.

There was a positive linear relationship between modelled and measured NO_2 concentrations at the three test sites ($t = 3.3$, $p < 0.01$). At NC, the model estimated roadside NO_2 to be $15.5 \mu\text{g m}^{-3}$, under-estimating measured concentrations by 5 %. At SCP, where there were physical barriers alongside the road, the model estimated roadside concentrations of NO_2 to be $27.9 \mu\text{g m}^{-3}$, and at SCH where the grassland is located 38 m above the road, the model estimated concentration of NO_2 as $29.3 \mu\text{g m}^{-3}$. The model over-estimated NO_2 concentrations nearest the roadsides by 28 % and 8 % at SCH and SCP, respectively.

3.4.2. Road derived changes to the soil physical and chemical environment

Concentrations of Zn ($t = -3.7$, $p < 0.001$) and Pb ($t = -4.7$, $p < 0.001$) were highest at the roadside and declined logarithmically with distance away from the road (appendix 3.2). Concentrations of Pb and Zn were unrelated to traffic density ($p > 0.05$), although levels of Cu increased with traffic density ($t = 2.8$, $p < 0.05$) and there was a less than additive interaction between distance and traffic volume for Cu across the eight sites ($t = -3.0$, $p < 0.01$). The highest concentrations of Zn in the soil were measured at SCH, which is the site situated 32 m above the roadside (table 3.2). Maximum concentrations of Pb and Cu were measured at AR and LD respectively. Minimum roadside values for Zn, Pb and Cu were all measured at SCP, where there was a physical barrier between the road and the grassland. Of the remaining HMs, Cr ($t = 3.5$, $p < 0.001$), Ni ($t = 2.3$, $p < 0.05$) and V ($t = 3.1$, $p < 0.01$) declined nearer the road, and As, Cd and Co were unrelated to roadside proximity (all $p > 0.05$).

Table 3.2: Mean soil concentrations of Zn, Pb and Cu at each site measured nearest and farthest from the road. Dispersal barriers relate to a row of trees located between the road and the grasslands (trees) or that the site is raised above the height of the grassland (raised). Bold typeface highlights highest and lowest values. Means are derived from 3 replicates $\pm$ s.e.

Site	Roadside Proximity (m)	Dispersal barriers	Concentration in soil (mg kg ⁻¹)		
			Zn	Pb	Cu
AR	3	NA	60.7 $\pm$ 4.3	55.8 $\pm$ 5.1	15.1 $\pm$ 0.9
	103		51.1 $\pm$ 10.8	25.3 $\pm$ 3.1	10.7 $\pm$ 2.0
BH	3	NA	168.7 $\pm$ 18.4	48.7 $\pm$ 2.6	9.7 $\pm$ 0.3
	103		81.3 $\pm$ 15.2	35.4 $\pm$ 1.6	7.0 $\pm$ 0.7
LD	1	NA	124.1 $\pm$ 26.1	48.9 $\pm$ 0.9	20.0 $\pm$ 0.7
	101		37.4 $\pm$ 5.9	26.9 $\pm$ 2.0	8.9 $\pm$ 0.8
MD	1	NA	213.1 $\pm$ 50.2	53.0 $\pm$ 4.5	11.6 $\pm$ 1.0
	101		85.3 $\pm$ 4.7	40.1 $\pm$ 3.3	7.6 $\pm$ 0.7
NC	3	Trees	95.3 $\pm$ 24.2	45.0 $\pm$ 4.7	9.3 $\pm$ 1.3
	103		64.4 $\pm$ 10.6	27.0 $\pm$ 2.6	10.0 $\pm$ 3.7
SCH	32	Raised	257.0 $\pm$ 107.4	34.3 $\pm$ 3.2	12.5 $\pm$ 0.3
	132		51.4 $\pm$ 13.1	21.9 $\pm$ 6.0	6.5 $\pm$ 1.9
SCP	2	Trees	29.4 $\pm$ 1.3	28.8 $\pm$ 4.3	5.5 $\pm$ 0.7
	102		25.7 $\pm$ 1.36	22.1 $\pm$ 1.6	4.3 $\pm$ 0.2
SD	2	Trees	77.4 $\pm$ 6.7	42.9 $\pm$ 5.4	7.9 $\pm$ 0.7
	102		78.6 $\pm$ 3.6	39.4 $\pm$ 3.2	9.7 $\pm$ 1.2

Soil pH declined logarithmically with distance from roadsides ($t = -2.2$, $p < 0.05$), with mean values of 7.7 ± 0.1 at roadsides declining to 7.5 ± 0.2 , ~100 m away (figure 3.1). Traffic density was also associated with an increased roadside pH ($t = 3.3$, $p < 0.05$) rising from 7.4 alongside the road with the lowest traffic flow rate, to 8.2 alongside the most heavily trafficked site. There was no general relationship between soil moisture or salt content of the soil and roadside proximity across the eight sites ($p > 0.05$), however, soil salinity was 3, 1.5 and 3 times higher at the first two location markers (nearest the road and then 2.5 m) compared with the remainder of the transect at BH, LE, NC, respectively.

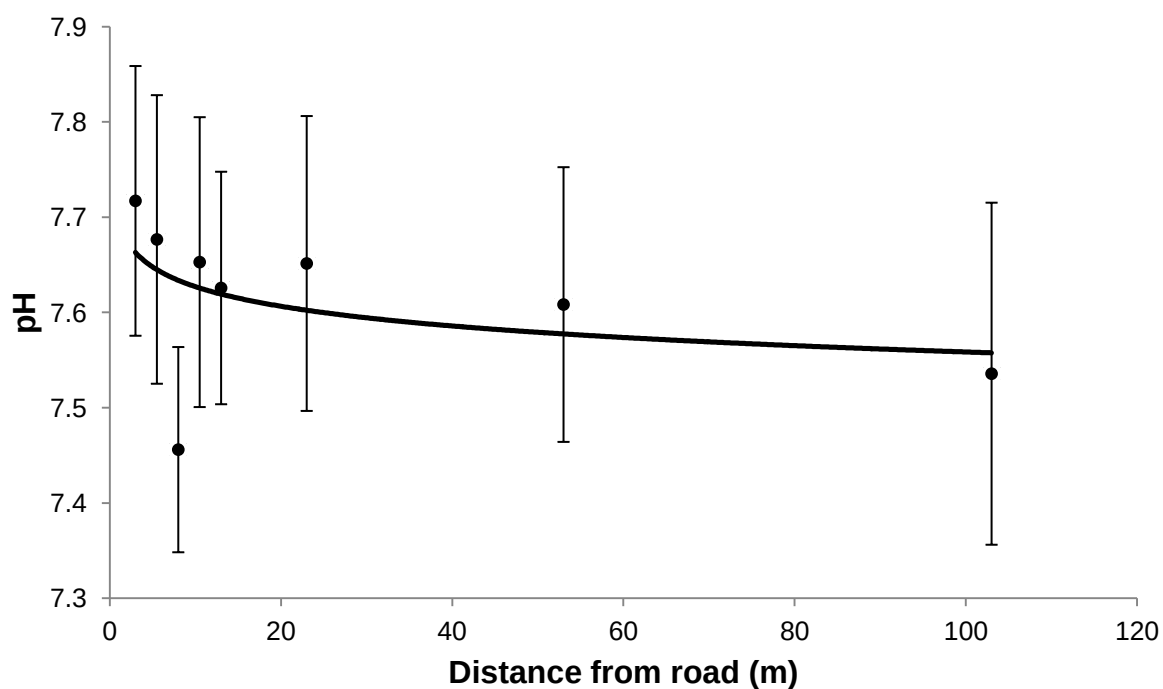


Figure 3.1: Relationships between pH and road proximity ($p < 0.05$). Values represent means across seven sites $\pm$ s.e.

3.4.3. Ellenberg scores, species richness and functional group shifts

There was an increase in frequency-weighted Ellenberg scores nearer to roadsides, declining logarithmically further from the road, for N ($t = -2.2$, $p < 0.05$), pH ($t = -2.1$, $p < 0.05$) and moisture ($t = -2.1$, $p < 0.05$) but not for salt ($p > 0.05$). At the individual site level, six of the eight sites displayed a decline in Ellenberg N scores away from the roadside whilst at two sites Ellenberg N scores increased away from the road (Figure 3.2). There was a positive logarithmic relationship between modelled dry NO_2 deposition and Ellenberg N scores ($t = 4.9$, $p < 0.001$, figure 3.3), however, Ellenberg N scores were not related to modelled dry NH_3 deposition, total dry N deposition or total dry + wet N deposition (all $p > 0.05$). There was a proportionally larger increase in Ellenberg N scores associated with lower dry NO_2 deposition rates; Ellenberg N scores increased by 19% when dry NO_2 deposition doubled from 2 to 4 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ whereas scores increased by only 3% as dry NO_2 deposition increased from 8 to 10 $\text{kg N ha}^{-1} \text{ yr}^{-1}$. Ellenberg pH, moisture and salt scores were not significantly related to measured pH, moisture or salt values (all $p > 0.05$).

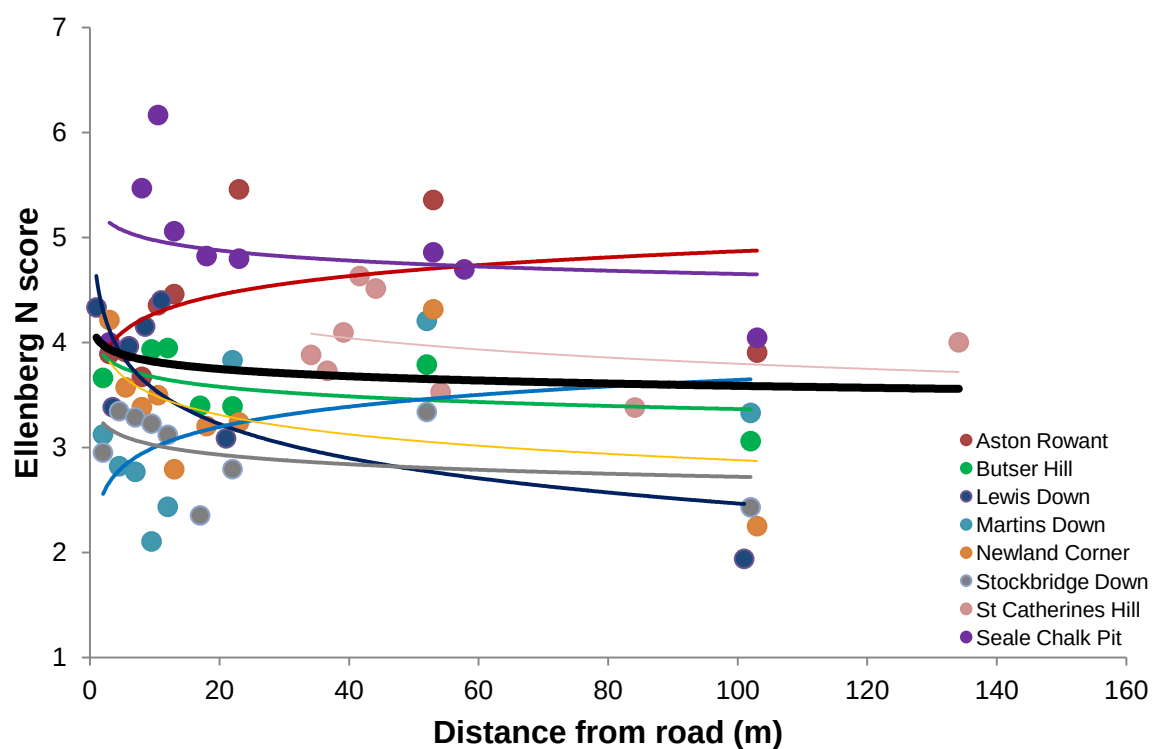


Figure 3.2: Relationship between frequency-weighted Ellenberg N indicator scores and distance from the road ($p < 0.05$) as described by the LME model (black line). Site specific relationships are also given (see figure legend).

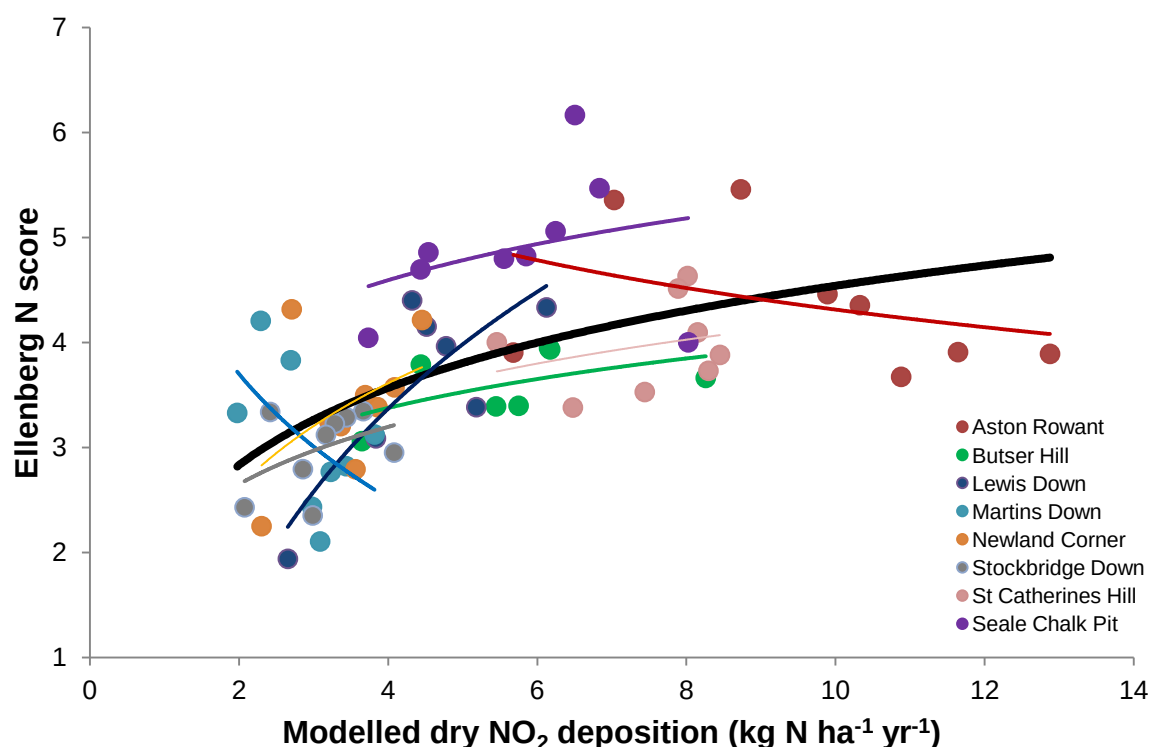


Figure 3.3: Relationship between frequency-weighted Ellenberg N indicator scores and modelled dry NO₂ deposition ($p < 0.001$) as described by the LME model (black line). Site specific relationships are also given (see figure legend).

Forb cover and forb species richness increased with modelled dry NO₂ deposition (table 3.3). Increasing modelled dry NO₂ deposition from 2 kg N ha⁻¹ yr⁻¹ to 12 kg N ha⁻¹ yr⁻¹ was associated with a mean increase in forb cover by 70 % and a mean increase in forb species richness by 2 m⁻². The increasing effect of modelled NO₂ deposition on forb species richness and forb cover was limited by moisture, and there was a significant less-than-additive interactive effect between moisture and NO₂ deposition. The relationship between NO₂ deposition and increasing forb abundance was driven by increases in the abundance of typical edge species ($t =$

3.7, $p < 0.001$) whereas the abundance of typical calcareous species was not related to any of the modelled parameters ($p > 0.05$).

Table 3.3: Summary of the minimal adequate model (MaM) for the LME (cover) and GLM (species richness) analyses across all sites. Terms in initial model: moisture (M), modelled dry NO₂ deposition (NO₂ dep), modelled dry NH₃ deposition, total N deposition (dry + wet) and their interactions (:). pH was not significant in any model and was omitted. Log denotes that logarithmic transformation was required to obtain the model which best fit the data. The remaining terms are untransformed.

Variable	MaM	Value	df	t/z	p
Grass cover	Intercept	80.4	52		
	log(M)	19.3		2.5	< 0.05
Forb cover	Intercept	21.7	50		
	M	3.8		1.5	ns
	NO ₂ dep	23.5		3.3	< 0.01
	M:NO ₂ dep	-0.7		-2.7	< 0.01
Shrub cover	Intercept	0.4	51		
	log(M)	-0.2		-2.4	< 0.05
	NO ₂ dep	0.7		2.3	< 0.05
Grass species richness	Intercept	2.5	60		
	Moisture	0.1		4.5	< 0.001
Forb species richness	Intercept	5.2	60		
	M	-0.1		-0.5	ns
	NO ₂ dep	1.5		5.1	< 0.001
	M:NO ₂ dep	-0.1		-2.5	< 0.05
Shrub species richness	na				ns
Total species richness	na				ns

Grass cover and species richness increased with increasing soil moisture, however, there was not a general relationship between soil moisture and roadside proximity. Grass cover increased by a mean of 50 % and grass species richness increased by a mean of 2 m⁻² when soil moisture increased from 4 % to 45 % across the survey locations. Cover of typical edge grass species increased with soil moisture content ($t = 2.5$, $p < 0.05$) whereas the cover estimates of typical calcareous grassland species were unrelated to any of the modelled parameters ($p > 0.05$).

3.4.4. Responses of individual plant species

Across the eight sites, three invasive species (as per the PLANTATT database) were identified: *Myosotis arvensis* (LD) and *Viola arvensis* (NC, SCP) which were only located within 5 m of the roadside, and *Geranium dissectum* (AR, LD, MD, SCP) which, when present, was found beyond 5 m from the roadside. There were also six shrub species: *Alnus incana* (NC), *Prunus spinosa* (SCP) and *Euonymus europeaus* (NC) which were only present within 5 m of the roadside. The shrubs: *Berberis vulgaris* (MD), *Cretaeus monogyna* (NC, SCP) and *Fraxinus excelsior* (SCP) were also found, but were not limited to a maximum roadside proximity.

There were three grass species and 11 forb species which increased in abundance nearer roadsides (table 3.4). Four of these species were typical edge species (*Holcus lanatus*, *Clinopodium vulgare*, *Geranium molle* and *Pastinaca sativa*), but none of the plant species which declined at roadsides were designated as edge species. Two grass species (*Dactylis glomerata* and *Holcus lanatus*) and two forb species, (*Geranium molle* and *Odontites vernus*) increased at roadsides and are classified as both N-tolerant and hydrophilic. An N-tolerant forb (*Pastinaca sativa*) and a hydrophilic forb (*Succisa pratensis*) also increased at roadsides.

Of the seven species which declined in abundance nearer roadsides, two grass species (*Koeleria macrantha* and *Trisetum flavescens*) and two forb species (*Picris hierarchoides* and *Sanguisorba minor*) are classified as poor competitors under high N and high soil moisture conditions. *Anagallis arvensis* and *Epilobium hirsutum* declined nearer the road but are classified as N-tolerant and hydrophilic. N tolerant *Lathyrus pratensis* also declined nearer roadsides. There were no relationships between any metallophytic or halophytic plant species and roadside proximity and all of the species which showed a significant relationship between abundance and roadside proximity were classified as pH tolerant.

Table 3.4: LME model output across all study sites to show the plant species which increased/decreased in abundance nearer to roadsides. Species with Ellenberg scores for pH, moisture (M) and nitrogen (N) above 5 are indicated with an “x”. Species which are categorised as typical calcareous (C) and typical edge species (E) are also indicated with an “x”.

Species	Functional group	t	p	M	pH	N	C	E
Increasing abundance nearer roadsides								
<i>Bromus erectus</i>	Grass	-2.3	< 0.05		x		x	
<i>Dactylis glomerata</i>	Grass	-2.5	< 0.01	x	x	x		
<i>Holcus lanatus</i>	Grass	-2.3	< 0.05	x	x	x		x
<i>Agrimonia euphatoria</i>	Forb	-2.0	< 0.05		x			
<i>Clinopodium vulgare</i>	Forb	-2.6	< 0.05		x		x	x
<i>Daucus carota</i>	Forb	-2.5	< 0.01		x			
<i>Filipendula vulgaris</i>	Forb	-2.1	< 0.05		x		x	
<i>Geranium molle</i>	Forb	-2.3	< 0.05	x	x	x		x
<i>Leontodon hispidus</i>	Forb	-2.1	< 0.05		x		x	
<i>Odentities vernus</i>	Forb	-2.1	< 0.05	x	x	x		
<i>Origanum vulgare</i>	Forb	-2.6	< 0.05		x			
<i>Pastinaca sativa</i>	Forb	-3.1	< 0.01		x	x		x
<i>Succisa pratensis</i>	Forb	-2.0	< 0.05	x	x			
<i>Thymus polytrichus</i>	Forb	-2.4	< 0.05		x			
Decreasing abundance nearer roadsides								
<i>Koeleria macrantha</i>	Grass	2.4	< 0.05		x		x	
<i>Trisetum flavescens</i>	Grass	2.4	< 0.05		x			
<i>Anagolis arvensis</i>	Forb	2.4	< 0.05		x	x		
<i>Epilobium hirsutum</i>	Forb	3.2	< 0.01	x	x	x		
<i>Lathyrus pratensis</i>	Forb	2.7	< 0.05	x	x	x		
<i>Picris hierarchoides</i>	Forb	2.5	< 0.05		x			
<i>Sanguisorba minor</i>	Forb	3.4	< 0.01		x		x	

3.5. Discussion

3.5.1. Modelling gradients of pollution deposition away from roadsides

The model used a logarithmic function to describe NO₂ declines away from roads, and a linear function to account for higher inputs of NO₂ when there is more traffic (Lee *et al.*, 2012). This approach was supported by a positive linear relationship between modelled and measured NO₂ concentrations at three test sites, indicating that the shapes of the curves which represent the decline in NO₂ concentrations away from roadsides were properly described.

There was good agreement between modelled and measured NO₂ at the site where there were minimal barriers to dispersal between the road and the grassland (NC). At the other two test sites, overestimation of NO₂ indicates that pollutant deposition may have been diminished by trees positioned between the road and the grassland at SCH, and also when the site was raised above the road at SCP. Over-estimation of NO₂ at SCH and SCP by 8 % and 28 %, respectively, is greater than the 3 % reduction in NO₂ as the result of roadside vegetation measured elsewhere (Yin *et al.*, 2011). Other factors such as the prevailing wind direction may be playing a role (Gilbert *et al.*, 2003). Study transects at NC, SCH and SCP were north, east and south of their adjacent roads, respectively. Despite these inaccuracies, the model adequately described the pattern and rate of NO₂ declines away from roads at all three test sites.

NO₂ was modelled to decline across the 100 m transects, with the model indicating that NO₂ may continue to decline 1,000 m from roadsides at some sites, supporting measurements taken elsewhere (Glasius *et al.*, 1999, Gilbert *et al.*, 2003). Modelled NO₂ deposition rates were considerably higher than background deposition rates at

all survey locations. It is therefore likely that NO₂ deposition rates continued to decline further from the roadside than the 100 m transects used in this study. In contrast, NH₃ was modelled to decline to negligibly above background concentrations within 20 m of roadsides. The high solubility of nitric oxide (NO), which was not modelled in this study, and its oxidation to form NO₂ means that NO declines to ambient concentrations at a similar rate to NH₃ (Cape *et al.*, 2004). Exhaust products with a lower deposition velocity may persist further from roadsides, for example elevated PAH concentrations have been measured 60 – 80 m from road edges (Viskari *et al.*, 1997). Other unmeasured pollutants (e.g. carbon dioxide, ozone) are present in relatively low concentrations have low deposition velocities therefore their influence on roadside vegetation may be minimal (Sehmel, 1980, Bignal *et al.*, 2007).

3.5.2. Edaphic contribution to roadside plant compositional change

Soil pH increased nearer roadsides likely due to dispersal of basic sodium containing compounds during weathering of road surfaces and subsequent run-off to the roadside environment (DfT, 2008). Ellenberg pH scores were not related to measured pH values indicating that road-derived pH increases may not be a key driver of change in adjacent calcareous vegetation. Dominance of plants that thrive in base rich and/or highly buffered soils (Bennie *et al.*, 2006), in addition to alkaline road run-off, means that roadside calcareous grasslands may be less susceptible to the acidifying effects of vehicle derived N deposition (Carroll *et al.*, 2003) than, for example heathland, which is characterised by weakly buffered acidic soil (Angold, 1997).

Pb and Zn increased nearer roadsides across the calcareous grasslands declining to background concentrations approximately 20 m and 5 m from road edges,

respectively. Cr, Ni and V declined nearer to roadsides but have been demonstrated to be mainly of natural origin at roadsides elsewhere (Pagotto *et al.*, 2001). High Zn concentrations measured at SCH may represent a legacy of chronic air pollution (Laschober *et al.*, 2004), since this elevated site did not receive road run-off. Low HM concentrations at SCP, where the adjacent road traffic density was the third highest of all the sites, are likely to be due to inhibition of dispersal by bordering trees and shrubs (Yin *et al.*, 2011). Associations between traffic density effects and soil Cu indicates that Cu may also be vehicle-derived (Harrison & Johnston, 1985). Soil salinity did not display a general relationship with roadside proximity across all sites but did increase nearer roadsides at three of the sites (BH, LD, NC) likely due to winter road salting or road re-surfacing (DfT, 2008).

Despite elevated HM concentrations across the sites and elevated soil salinity at some sites, the lack of relationships with metallophytic and halophytic plant species may be explained by a rapid decline in soil salinity away from the road edge (Thompson *et al.*, 1986), immobilisation of HMs within base-rich calcareous soils (Martínez & Motto, 2000) and/or soil HM and salt concentrations being sufficiently low to prevent ecological changes from occurring. Inhibition of pollutant dispersal by bordering vegetation may have contributed to low HM and salt concentrations nearer roadsides at some sites. The beneficial effect of road-bordering vegetation in inhibiting pollutant dispersal may be partially offset by the appearance of some shrubs and invasive species, within the first 5 m from road edges, which are likely to have been propagated from the bordering vegetation.

3.5.3. Identifying the key drivers of roadside plant compositional changes

The general trend of declining frequency-weighted Ellenberg N scores further from roadsides indicates that N enrichment may be a driver of roadside vegetation

changes (Stevens *et al.*, 2004, Van den Berg *et al.*, 2011) providing support for the findings from two calcareous grasslands (Lee *et al.*, 2012). Furthermore, Ellenberg N scores were related to dry NO₂ deposition rates, but not other forms of dry and wet N deposition, indicating that NO₂ from vehicle exhausts is a key driver of roadside plant compositional change. The forbs *Filipendula vulgaris* and *Thymus polytrichoides* also increased nearer roadsides and have been demonstrated to increase in response to experimental N addition in a calcareous grassland ecosystem (Wilson *et al.*, 1995). Increased abundances of five nitrophilic plant species nearer roadsides (*Dactylis glomerata*, *Holcus lanatus*, *Geranium molle*, *Odentites vernus* and *Pastinaca sativa*) provides further support of N-driven compositional change. Ellenberg indices did not decline further from the roadside at two of the eight sites indicating that other environmental variables (e.g. soil moisture or pH) may be playing a role.

Increases in the abundances of forbs with increasing NO₂ deposition were not anticipated, however across this multi-site study, increases in forb abundances were driven by taller, faster growing edge species and not typical calcareous species. Presumably edge species are better able to assimilate additional N and outcompete subordinate calcareous species (Williams *et al.*, 2005). Grasses proliferated in calcareous soils of higher moisture content with this relationship also being driven by increases in the abundance of typical edge species rather than calcareous species. The less-than-additive interactive effect between moisture and NO₂ deposition on forb abundances indicates that hydrophilic species (especially grasses) may be proliferating near roadsides in soils of high moisture content despite high NO₂ deposition rates, where increased abundances of nitrophilic species may also be expected.

There was a greater relative increase in Ellenberg scores at relatively low NO₂ deposition rates, between 2 and 4 kg N ha⁻¹ yr⁻¹, than at higher NO₂ deposition rates between 8 and 10 kg N ha⁻¹ yr⁻¹. CLE for NO_x for the protection of vegetation and of ecosystems translates to an N deposition rate of approximately 9 kg N ha⁻¹ yr⁻¹. One explanation for plant compositional changes at low N inputs in calcareous grasslands is that nutrients other than N (e.g. phosphorus) become limiting as N deposition rates increase (van der Hoek *et al.*, 2004), or alternatively the most sensitive plant species are outcompeted at low N inputs (Critchley *et al.*, 2002). Although it should be noted that CLOs relate to total N deposition and not just NO₂ deposition, these deposition rates are considerably lower than the CLO for calcareous grasslands of between 15 and 25 kg N ha⁻¹ yr⁻¹ (Bobbink & Hatteling, 2011).

Relationships between Ellenberg N scores and dry NO₂ deposition across the 100 m transects compliments documented N-driven plant compositional changes up to 100 m from the roadside in ombrotrophic bogs (Bignal *et al.*, 2007), 200 m in heathland (Angold, 1997) and 230 m in the ground flora of coniferous woodland (Bernhardt-Romermann *et al.*, 2007). Since few exhaust products and other environmental changes persist 100 m from roadsides, Ellenberg indicator scores, individual nitrophilic plant species and pollutant dispersal characteristics together provide evidence that NO₂ plays an important role in determining plant species compositional changes adjacent to roads. Contributions by other exhaust gases cannot, however, be discounted. In the UK, 5.4 % of SSSIs are located in an estimated region of road influence, within 200 m of a road (Weigert, 2004). If a similar proportion of calcareous grasslands are located within the region of road influence then plant species composition in 3,500 ha of calcareous grassland may be affected by road derived pollution (Countryside-Survey, 2008). With some studies demonstrating

elevated NO₂ concentrations at distances up to 1,000 m from roadsides (e.g. Glasius *et al.*, 1999) this estimated zone of influence may be a conservative estimate.

3.6. Conclusions

This multi-site study provides evidence that NO₂ deposition is an important determinant of compositional change in roadside calcareous plant communities. Nitrophilic plant species, species which are typical of habitat edges, invasive species and shrubs were more frequently observed at roadsides. Currently, calcareous grasslands are typically managed using grazing livestock, however, this management practise may not prevent the dispersal of vehicle derived pollutants or inhibit perturbations to hydrology and soil biogeochemistry. Since the ecological effects of roads may extend a substantial distance away from road edges, buffer zones and physical barriers alongside roads may prevent the dispersal of exhaust emissions and inhibit the surface run-off of rainwater, impeding the deposition of exhaust products and road surface chemicals nearby. If plants are used as physical barriers, care should be taken to select species which will not encroach into the calcareous grassland itself. With calcareous grasslands currently declining in coverage, understanding the relationships between roads, site topography, buffer zones, physical barriers and vegetation is important to inform management decisions aimed at arresting their decline and enhancing their conservation value as traffic volumes increase across many parts of Europe.

4. Plant and arthropod community sensitivity to climate change and nitrogen enrichment in a successional grassland ecosystem

4.1. Abstract

Grasslands provide many ecosystem services including climate regulation via carbon (C) sequestration, maintenance of genetic resources (biodiversity), agricultural pest control and the provision of food. Predictions of grassland responses to future climate change are equivocal, in part because relatively few empirical data are available characterising ecological responses to climate change, particularly in combination with other global changes such as nitrogen (N) enrichment. A mesotrophic grassland ecosystem in Southern England was exposed to simulated end-of-century rainfall regimes based on IPCC 4th Assessment projections (+ 15 % winter rainfall and – 30 % summer rainfall) or ambient climate. N was also added to half the experimental plots in a factorial combination. Plant species composition and above ground biomass was resistant to rainfall manipulation and N enrichment in the first two summers and resistance to N enrichment persisted throughout the three year experiment. In the third year, above-ground biomass declined in rainfall manipulated plots and there was a trend toward increasing forb abundance and declining grass abundance. Declines in plant productivity were associated with declines in the abundance of plant feeding *Auchenorrhyncha* and carnivorous *Araneae* in rainfall manipulated plots in the third year. Shifts in plant and arthropod functional diversity and plant biomass responses to rainfall manipulation were not

modified by N enrichment, a finding that may have important implications for C stocks, biodiversity preservation and crop yields.

4.2. Introduction

The global nitrogen (N) and carbon (C) cycles, and various aspects of climate are projected to be further altered by anthropogenic activity during the current century (IPCC, 2007, Galloway *et al.*, 2008, IPCC, 2008). As a result, many natural ecosystems including grasslands are likely to experience novel environmental conditions in the future, with unknown consequences for their capacity to provide the range of important ecosystem services (e.g. pollination, C-sequestration and agricultural pest management) that they currently provide (Sala *et al.*, 2000, Millennium Ecosystem Assessment, 2006). Predictions of the ability of grasslands to continue to provide ecosystem services are equivocal, in part because there is insufficient quantitative data describing plant responses to the range of global changes that are anticipated, and in part because it is not known how multiple global change factors will interact to modify these responses (Trumper *et al.*, 2009).

In 2000 11% of the world's natural vegetation received N deposition greater than 10 kg ha⁻¹ yr⁻¹, a deposition rate which would have been extremely rare in 1860 (Dentener *et al.*, 2006). Reactive N creation is continuing to rise, with substantial areas of the world projected to be exposed to N deposition greater than 50 kg ha⁻¹ yr⁻¹ by 2050 (Galloway *et al.*, 2004). Biomass gains in response to future increased N deposition are anticipated, since the productivity of many temperate forests, tropical forests, temperate grasslands, tropical grasslands, wetlands and tundra are limited by N availability (LeBauer & Treseder, 2008). A shift in resource allocation by plants may also be expected across grasslands in response to N enrichment, through increased above-ground growth and a reduction in root growth (Lee *et al.*, 2010).

Declines in regional and local biodiversity as well as shifts in plant and arthropod community composition are also associated with increased N deposition across national N gradients and N enrichment experiments (Stevens *et al.*, 2004, Stevens *et al.*, 2006, Bobbink *et al.*, 2010, Eisenhauer *et al.*, 2011, Stevens *et al.*, 2011).

Climate models are predicting that many of the world's ecosystems (particularly northern temperate zones) will experience an increased frequency of drought events (IPCC, 2008). Grasslands are of particular interest because there is evidence that there is a greater response in the annual net primary productivity of grasslands to variability in rainfall than is found in other biomes (Knapp & Smith, 2001).

Experimental manipulation of rainfall regimes and monitoring of grassland plant community responses to natural drought periods has demonstrated that reduced plant biomass production (and hence C-sequestration), changes to species composition and localised species extinctions are symptomatic of reductions in rainfall (Tilman & Haddi, 1992, Laporte *et al.*, 2002, Morecroft *et al.*, 2004). There is substantial variation between study sites though, with biomass declines in some grasslands occurring within one year of reduced rainfall (Tilman & Haddi, 1992) whereas the productivity of other grasslands remains resistant for a decade or more (Grime *et al.*, 2000).

Arthropod communities may also influence terrestrial C-stocks and plant species richness if herbivore abundance is a key determinant of plant biomass production and turnover (Gough *et al.*, 2007). Declines in the abundance of phloem feeding insects have been associated with rainfall reductions as feeding becomes inhibited by reduced cell turgor and plant water content (Mattson & Haack, 1987, Huberty & Denno, 2004). Increases in the abundance of carnivorous arthropods (spiders) and increases in the abundance of herbivorous sap-feeding arthropods have been

associated with N enrichment, with most of the variation associated with increased plant productivity (Boyer *et al.*, 2003, Cole *et al.*, 2008). Other N addition studies have demonstrated suppression of herbivore abundances by increases in predators as habitats become more suitable for hunting (Fraser & Grime, 1998). There is tentative evidence that drought and N enrichment may have an interactive effect on the arthropod community, since increased abundances of plant feeding insects have been associated with drought and increases to plant tissue N content (Lauenroth *et al.*, 1978), though tissue N content was increased by water stress rather than N enrichment in this case.

Plant biomass production in response to N enrichment has been demonstrated to be dependent on the degree of N enrichment and global climate variability, with a meta-analysis showing reduced biomass responses to N enrichment in regions of the world with low rainfall (Lee *et al.*, 2010). Despite the interactive effect of climate and N, few studies have experimentally investigated the responses of grasslands to N enrichment and manipulated rainfall regime simultaneously. One such study documented above-ground productivity increases in a grassland only occurred when water and N were added together (Harpole *et al.*, 2007). Another study demonstrated greater than additive plant biomass gains in response to water and N addition in a semi-arid grassland (Lauenroth *et al.*, 1978). However, there are no studies in grasslands which have investigated the effects of both a reduction in rainfall and N enrichment simultaneously (Bobbink *et al.*, 2010). This has only been investigated in heathland, where N addition and rainfall reduction together increases community susceptibility to invasion by grasses (Bobbink *et al.*, 1998).

This study sought to contribute to this knowledge gap by simulating projected 2100 rainfall patterns and future N enrichment, both individually and in combination in a

mesotrophic grassland in the UK measuring a range of ecosystem properties (some community, some biogeochemical). The following hypotheses were tested: (1) soil moisture content, plant productivity, herbivorous insects and their predators would decline in rainfall manipulated plots in response to summer drought, with increases in the abundance of drought tolerant plant species, (2) soil N availability and plant productivity would increase in N enriched plots with associated increases in nitrophilic plant and sap sucking arthropod species and (3) plant biomass declines, and plant and arthropod community shifts in response to rainfall manipulation would be reduced or prevented by N enrichment when both rainfall manipulation and N enrichment were applied together.

4.3. Materials and methods

4.3.1. Site and treatments

The study took place in a lowland mesotrophic grassland, MG6: *Lolium Perenne* / *Cynosaurus cristatus* (Rodwell, 1992) on loamy sand soil at Silwood Park, Ascot, UK (51.507N, 0.640W). On commencement of the study the N content of the soil across all plots was relatively high ($7.1 \text{ mg kg}^{-1} \pm 0.1$). Mean annual temperature and rainfall over the four years ending December 2009 was 11 °C and 741 mm yr⁻¹, respectively. Summer 2011 (57 %) and winter 2009 (66 %) rainfall was considerably higher than the mean rainfall over the previous four years (table 4.1). The site was ploughed in October 2007, and then left undisturbed until commencement of the study in May 2009. Dominant plant species over the duration of the study were the grasses *Agrostis capillaris*, *Arrhenatherum elatius*, *Dactylis glomerata* and *Holcus mollis*, the forb *Cirsium arvense*, and the legume *Trifolium repens*. Site weather data were obtained using an onsite Vantage Pro weather station (Davis Instruments, USA).

Table 4.1: Summary of mean summer and winter rainfall received in the four years ending December 2009 prior to commencement of the study and throughout the study in ambient and rainfall manipulated plots.

Period	Ambient rainfall (mm)	Rainfall manipulated (mm)
Summer		
Prior	140.6	
2009	135.3	67.7
2010	134.2	92.1
2011	221.2	140.0
Winter		
Prior	167.6	
2009	278.2	312.7
2010	161.4	185.6

A fully factorial, randomised block design was used with 16, 2.4 m x 2.4 m plots (appendix 4.1). The use of four blocks accounted for any gradients in initial plant species composition and soil biogeochemistry as a result of site specific factors such as drainage and shading. Treatments were N enrichment (0 or 40 kg ha⁻¹ yr⁻¹, herewith N⁰ and N⁺) and rainfall manipulation (ambient climate or 2100 predictions, herewith referred to as rainfall manipulated) arranged in a factorial design with four treatments: (1) nitrogen enrichment only (ambient climate, N⁺), (2) nitrogen enrichment with predicted 2100 rainfall (rainfall manipulated, N⁺), (3) predicted 2100 rainfall only (rainfall manipulated, N⁰) and (4) control (ambient climate, N⁰).

Treatments were assigned at random within four replicate blocks, giving a total of 16 plots. Treatments commenced in May 2009 and continued until August 2011.

Rainfall manipulations simulated regional end-of-century rainfall projections (Murphy et al., 2009): Summer (JJA) rainfall is predicted to decline by 30% in South East

England, with an increased frequency of drought events. Winter (DJF) rainfall is predicted to increase by 15%. During the summer months only, rainfall manipulated plots were covered with transparent corrugated plastic roofs to collect all rainfall and ambient climate plots were covered with transparent corrugated plastic roofs with holes in to allow rain to pass through to the plots below (appendix 4.2). In rainfall manipulated plots all collected rainfall was applied if more than 20 mm fell over a 24 hour period, and 50% of rainfall was applied if less than 20 mm fell. This was designed to emulate projected stochasticity in rainfall events, by creating a regime of small inputs, interspersed with large downpours aiming to reapply ~70% of rainfall collected. Over the three summers an average of 61% of summer rainfall was reapplied (2009 – 50%, 2010 – 69%, 2011 – 63%), slightly less than target. In winter, plots receiving the rainfall manipulation treatment received an additional 15% of rainfall (collected adjacent to plots). In winter and summer, rainfall was re-applied manually following each rainfall event using a watering can and rose nozzle to ensure even dispersal across each plot. Over the three years, rainfall manipulated plots received a mean of 91% of the annual rainfall received by ambient climate plots (appendix 4.3).

Annual background deposition rates at the study site have been modelled to be 18 kg ha⁻¹ y⁻¹ (RoTAP, 2011) hence total N deposition in N enriched plots was estimated to be 58 kg ha⁻¹ y⁻¹. Nitrogen, in the form of ammonium nitrate, was applied twice monthly at a rate of 40 kg ha⁻¹ y⁻¹ to N⁺ treatment plots. This is the upper end for N deposition rates received by European non-forest ecosystems in 2000 (Dentener *et al.*, 2006). Ammonium nitrate was added to plots dissolved in 5L of rainfall, which had been collected onsite (concentration = 7mMol) to minimise the amount of additional water added per plot. 5L of rainfall with no additional N was added

concurrently to N^0 plots to ensure consistency of the rainfall received by N^+ and N^0 plots.

4.3.2. Soil moisture, extractable nitrogen and mineralisation

PVC cores (2.5 cm diameter, 15 cm length) were used to collect soil samples at six time intervals: June 2009, September 2009, December 2009, March 2010, June 2010 and June 2011, from each plot with sampling designed to monitor seasonal fluctuations by season. PVC cores were immediately reintroduced into the ground adjacent to the original sampling location, covered to prevent leaching losses and left for three months before the soil was collected (incubated sample). Extractable inorganic oxidised nitrogen ($NO_2^- + NO_3^-$) and reduced nitrogen (NH_4^+) were extracted from a 10 g sub-sample of incubated and fresh soil by mixing with 37.5 ml of 1 M KCl and concentrations were measured using a SKALAR San Flow⁺⁺ auto-analyser (SKALAR, Netherlands). A further 5 g sub-sample was dried in a drying oven for 48 hours ($80\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$), with the relative mass loss taken as soil moisture content. SKALAR outputs were adjusted for soil moisture content to give extractable N concentrations per unit dry soil (mg kg^{-1}). Spot measurements of soil moisture were made using a ThetaProbe soil moisture sensor (Delta-T, UK).

Mineralisation rates (MR) and nitrification rates (NR) were calculated for summer (June 2009 – September 2009), autumn (September 2009 – December 2009), winter (December 2009 – March 2010) and spring (March 2010 – June 2010) by comparison of extractable N content of incubated (t_2) and fresh soil samples (t_1) using formulae 1 and 2 below. The sum of mineralisation rates and nitrification rates from each of the four periods gave annual mineralisation and nitrification rates in the year ending June 2010 ($\text{g m}^{-2} \text{ yr}^{-1}$). Bulk density was calculated as the total dry mass of soil, divided by the volume of the PVC cores (volume = 73.6 cm^3).

- (1) $MR = \text{total extractable N } (t_2) - \text{total extractable N } (t_1) * \text{bulk density}$, and
- (2) $NR = \text{extractable oxidised N } (t_2) - \text{extractable oxidised N } (t_1) * \text{bulk density}$.

4.3.3. Plant composition and biomass

Cover estimates of vascular plant were made using 1m x 1m quadrats, placed in the central 1 m² of each plot. Vascular plants were identified to species level and percentage cover of all vascular plants was recorded. The cover abundance of individual species of grasses, forbs and legumes were added together to give cover estimates for the three functional groups. Vegetation surveys took place in July 2009, July 2010 and July 2011.

Destructive harvests were carried out in September 2011 when many species were at maximum annual biomass. Vegetation from four 25 cm x 25 cm randomly positioned sub-samples (representing 25 % of the central 1 m² plot area) was cut to ground level and removed from all 16 plots. Samples were dried for 48 hours, litter was removed and species were identified and weighed. In September 2009, 2010 and 2011 vegetation height was used as a proxy for above-ground biomass by measuring plant height at 50 locations across each plot.

Root growth was measured by installation of 4 nylon mesh cores (780 µm diameter meshes, 15 cm length, 2.5 cm width) in May 2010 and May 2011, containing sieved soil which had been collected from each plot on the same day as they were introduced. Sieving of the soil removed roots prior to introduction into the plots.

Root cores were removed in July 2010 and July 2011. Roots that had grown into the cores were extracted, washed, dried and weighed.

4.3.4. Arthropod community

Arthropod sampling took place during a one-week period in July 2010 and 2011. All samples were collected between 12pm and 3pm on dry days to ensure conditions favoured a consistent arthropod community between surveys. Arthropods were collected using a Vortis suction sampler and frozen. In each plot, sampling took place four times at random locations within the central 1 m² of each plot with a suction duration of 16 s, which has been demonstrated previously to collect 90% of the invertebrates present in grassland (Brook *et al.*, 2008). Four replicates within each plot represented a sampling coverage of 16% of the central 1 m² of each plot. Species were identified to order (*Hemiptera* and *Arachnida* were identified to sub-order) and counted.

4.3.5. Statistics

Power analyses were used to assess the statistical power of two key variables, above- and below-ground biomass using within and between group variance based on data obtained in year one (appendix 4.4). Extractable N, mineralisation rates, nitrification rates, plant cover estimates, insect abundance counts, above- and below-ground biomass and plant and insect species diversity were statistically tested for significance using two-way ANOVA (v2.11.1, www.r-project.org). Data were transformed to achieve normality prior to statistical testing where appropriate. Climate and N treatments and the interaction between both variables were introduced into the initial statistical models alongside block. The influence of blocking was retained within ANOVA models to ensure treatment effects were not driven by block. Removal of the highest order non-significant term continued until the minimum adequate model was produced, with the validity of removal of model terms tested using a likelihood ratio deletion F test (Crawley, 2007).

4.4. Results

4.4.1. Soil biogeochemistry

Soil moisture content in rainfall manipulated plots was lower than ambient climate plots within two weeks of commencement of the summer treatments (figure 4.1).

Annual mineralisation rates in the survey year ending June 2010 were increased by 50 % in N^+ plots compared with N^0 plots (figure 4.2a), but differences between treatments were not significant ($p > 0.05$, table 4.2). Elevated mineralisation in N^+ plots was associated with increased annual nitrification rates, with nitrification rates in N^+ plots increasing by 90 % compared with controls ($f = 4.6$, $p < 0.01$, figure 4.2b). Annual nitrification comprised 70 % of annual mineralisation rates across the plots. Whilst annual nitrification rates showed significant treatment effects, there were no significant treatment effects on mineralisation or nitrification rates during any of the quarters when analysed individually (all $p > 0.05$). No interactive effects between rainfall and N were identified (all $p > 0.05$).

Table 4.2: ANOVA analyses summarising plant responses to nitrogen (N) or rainfall manipulation (Rainfall) when compared with controls (No N and ambient climate). Direction of responses denoted as increasing (↑) or decreasing (↓). Details given if $p \leq 0.10$.

There were no significant interactive effects identified in the analyses and hence these are not stated.

Response	Period	2009			2010			2011		
		Treatment	f	p	Treatment	f	p	Treatment	f	p
Soil	Available oxidised N	Summer	ns							
		Autumn	↑ N	4.0	0.07					
		Winter	ns							
		Spring	ns							
	Available reduced N	Summer	ns							
		Autumn	ns							
		Winter	ns							
		Spring	ns							
Vegetation	Grass cover	Summer	ns					↓ Rainfall	3.5	0.08
	Forb cover	Summer	ns					↑ Rainfall	3.2	0.10
	Grass cover : forb cover	Summer	ns					↓ Rainfall	3.0	0.10
	Species richness	Summer	ns					ns		
	Grass biomass	Summer						↓ Rainfall	11.4	0.001
	Forb biomass	Summer						ns		
	Total biomass	Summer						↓ Rainfall	12.1	0.001
	Vegetation height	Summer	ns					↓ Rainfall	2.7	0.10
	Root biomass	Summer	ns		↑ Rainfall	3.6	0.09	ns		

In terms of spot measurement of extractable N, oxidised N was elevated in N^+ compared with N^0 plots in autumn 2009 but was not significant ($p > 0.05$).

There were no significant treatment effects on available oxidised N at any other time (all $p > 0.05$). There were no treatment effects on available reduced N during the entire survey period (all $p > 0.05$).

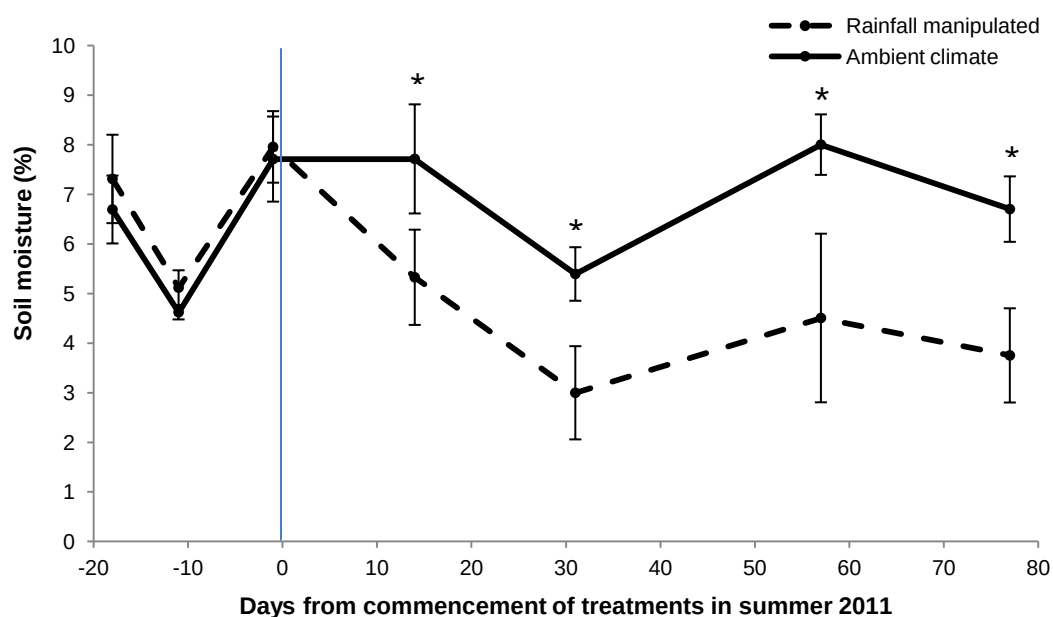


Figure 4.1: Soil moisture (%) over time in summer 2011 in the rainfall manipulated and ambient climate treatments. Values are the means of 3 measurements taken from each of the 8 rainfall manipulated and 8 ambient climate plots at each time point. The vertical line represents commencement of the summer treatments. * denotes $p < 0.05$.

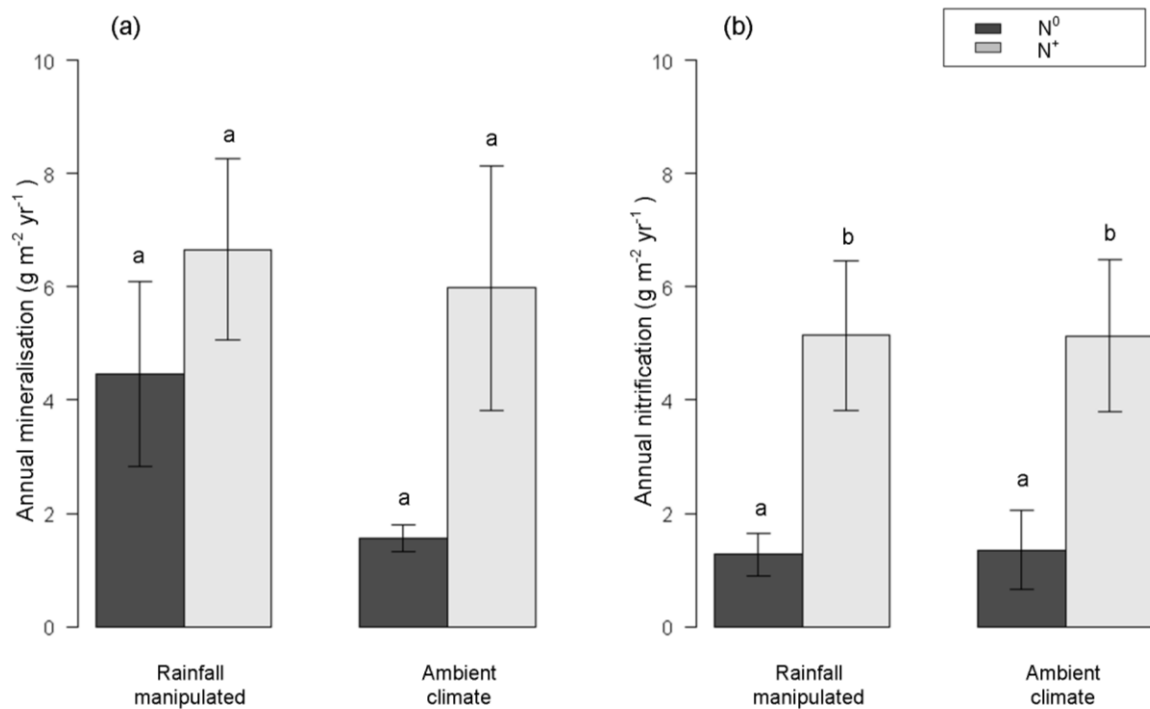


Figure 4.2: Annual (a) mineralisation rates (b) nitrification rates across each of the four treatments. Values are the means of four replicates. Letters a and b denote significantly different treatments at $p < 0.05$.

4.4.2. Species richness, plant composition and biomass production

Grass biomass comprised the vast majority of total plant biomass (~96 %) across all plots. Across all plots mean vegetation height, which was used as a proxy for aboveground biomass, was 9.9 cm, 10.7 cm and 13.2 cm in years 1, 2 and 3, respectively. Differences in vegetation height, between rainfall manipulated plots and ambient plots, translated to 3 %, - 0.3 % and - 14 % in years 1, 2 and 3 respectively. Though treatment effects were not significant across all three years (all $p > 0.05$), there was a trend towards significance over the treatment years with p declining to 0.1 by year 3 (Table 4.2).

In the third treatment year (2011), total above-ground biomass was also assessed by harvesting and was shown to decline significantly by a mean of 28 % in rainfall manipulated plots compared with ambient climate plots ($p < 0.01$). This was driven by a decline in grass biomass ($p < 0.001$, table 4.2) as forb biomass was unaffected by any of the treatments ($p > 0.05$). N enrichment did not have a significant effect on above-ground biomass production ($p > 0.05$), however, power analyses revealed differences in the explanatory power of the dataset for N (0.05), rainfall manipulation (0.29) and the interaction between N and rainfall manipulation (0.20).

Below-ground, mean root growth across all plots was 1.5 g cm^{-2} , 1.6 g cm^{-2} and 3.5 g cm^{-2} in years 1, 2 and 3, respectively. There was a 0.5 g cm^{-2} (34 %) increase in root growth in rainfall manipulated plots compared with ambient climate plots in the second treatment year, though this was not significant ($p > 0.05$). In the first and third treatment years, there was a relatively small increase in additional root growth in rainfall manipulated plots; 0.2 g cm^{-2} (14 %) and 0.3 g cm^{-2} (9 %), respectively (all $p > 0.05$). N enrichment did not result in any changes to above- or below-ground biomass accumulation throughout the experiment ($p > 0.05$). There were also no interactive effects between rainfall and N enrichment identified (all $p > 0.05$). Lower statistical power of the dataset was found for below-ground root growth for N (0.11), rainfall manipulation (0.05) and the interaction between N and rainfall manipulation (0.09).

Whilst there were no effects of treatment on the cover-weighted ratio of grasses to forbs or the cover of grasses or forbs in 2009 or 2010 (all $p > 0.05$), there was a decline in the grass:forb ratio in 2011 in rainfall manipulated plots though this was not significant ($f = 3.8$, $p > 0.05$, figure 4.3). This shift reflected a mean

9% decline in grass cover, and a mean 70% increase in the forb cover of rainfall manipulated plots when compared with ambient climate plots. Species richness of grasses and forbs were not related to any of the treatments in 2009, 2010 or 2011 (all $p > 0.05$). The decline in grass coverage in rainfall manipulated plots was driven by decreases in the dominant three species: *Agrostis capillaris*, *Arrhenatherum elatius* and *Holcus mollis* ($f = 4.7$, $p < 0.05$). However, the coverage of individual forb and grass species were not related to any of the treatments (all $p > 0.05$).

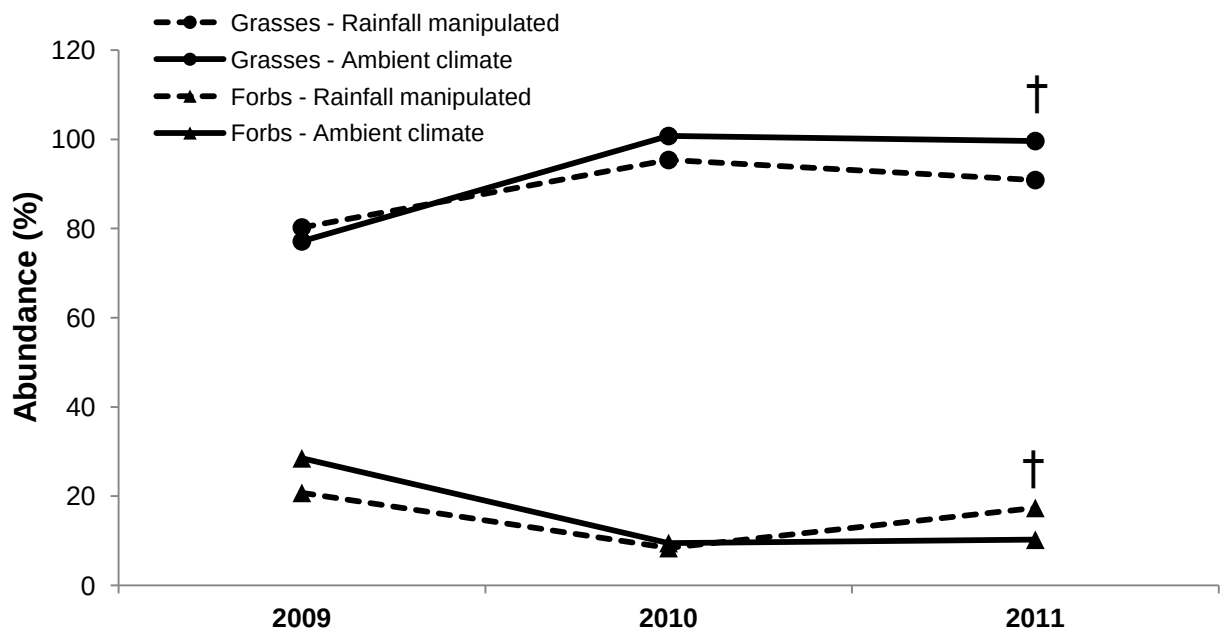


Figure 4.3: Mean cover (%) of grasses and forbs in rainfall manipulated and ambient climate treatment in summer 2009, 2010 and 2011 without standard error bars for illustrative purposes. Values are the means of eight replicates. † denotes near significance at $p \leq 0.1$.

4.4.3. Arthropod community

There were no treatment effects on any arthropod species in the second year in response to treatments (all $p > 0.05$ in 2010). In the third treatment year, however, the number of *Hemiptera* (*Auchenorrhyncha* + *Heteroptera*) individuals declined in rainfall manipulated plots ($f = 7.8$, $p < 0.05$). This was driven by a 40 % decline in the number of *Auchenorrhyncha* ($f = 8.0$, $p < 0.05$), with an average of $406 \text{ m}^{-2} \pm 46$ individuals in the ambient climate plots and $244 \text{ m}^{-2} \pm 36$ in the rainfall manipulated plots (figure 4.4a). *Heteroptera* abundance was not altered by rainfall manipulation or N inputs on any measurement occasion ($p > 0.05$).

Overall *Arachnida* (*Acari* + *Aranae* + *Opiliones*) abundance was not related to any of the treatments ($p > 0.05$). There was, however, a 33 % decline in the number of *Aranae* in rainfall manipulated plots ($f = 7.0$, $p < 0.05$), with an average of $75 \text{ m}^{-2} \pm 7$ individuals in the ambient climate plots and $50 \text{ m}^{-2} \pm 8$ in the rainfall manipulated plots (figure 4.4b). The abundance of *Acari* and *Opiliones* were not related to any of the treatments ($p > 0.05$). *Hymenoptera* increased by 12 % in N^+ plots, with a mean of $169 \text{ m}^{-2} \pm 19$ individuals in N^+ and $148 \text{ m}^{-2} \pm 36$ in N^0 plots (figure 4.4c). The abundances of individuals from other insect orders were not related to any of the treatments ($p > 0.05$).

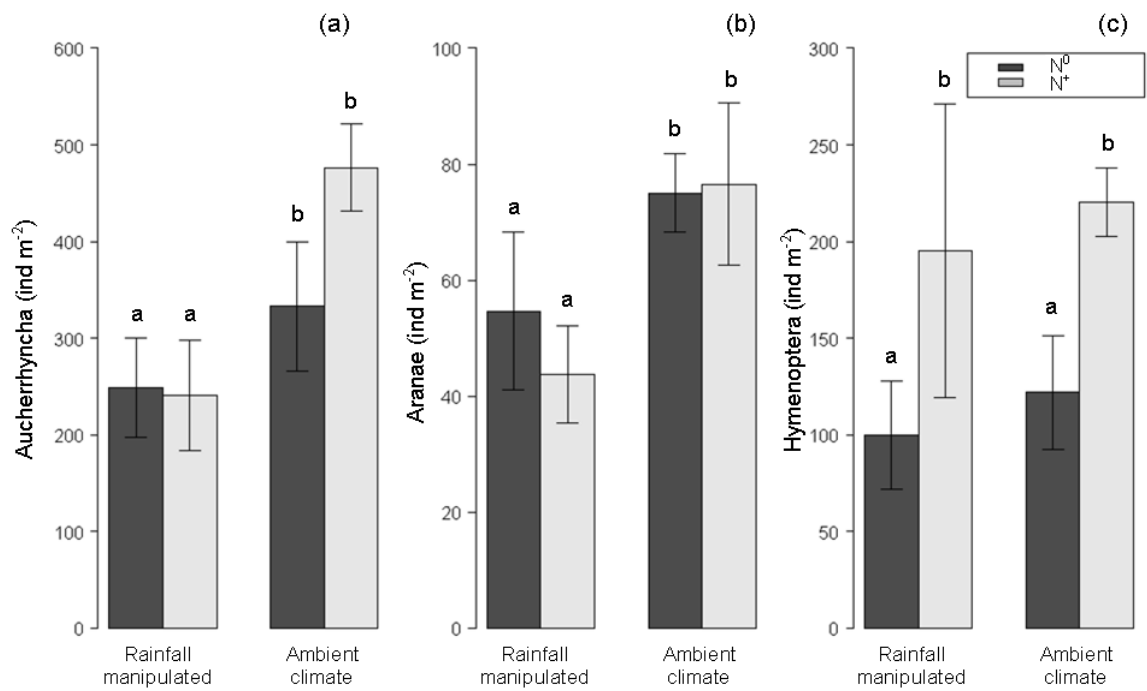


Figure 4.4: Individuals (ind) of (a) *Auchenorrhyncha*, (b) *Araneae* and (c) *Hymenoptera* across each of the four treatments. Values are the means of four replicates. Letters a and b denote significantly different treatments at $p < 0.05$.

4.5. Discussion

The grassland was shown to be largely unaltered by rainfall manipulation and N enrichment, both individually and in combination, in the first two years of the experiment. Rapid (< two years) plant productivity declines and changes in plant species composition in response to severe short-term drought (0% of rainfall re-applied in June and August) have been observed in early successional grassland (Grime *et al.*, 2000). In contrast, above-ground biomass and the relative coverage of plant functional groups did not respond to rainfall manipulation over the first two years in the mesotrophic grassland presented here. This complements previously demonstrated plant community resistance to drought (50 % of spring and summer rainfall reapplied) during the first three years in short-grass steppe, with productivity declines following drought treatment only occurring in the fourth and seventh years (Evans *et al.*, 2011).

One mechanism for above-ground plant community resistance during this initial period is by higher investment of plants resources below-ground to access the scarce water in the summer (Kalapos *et al.*, 1996). Root growth increased in rainfall manipulated plots in all years, but was only statistically significant in year two when the difference between the treatment means was at its maximum. Low explanatory power for the below-ground biomass dataset means that the effect size would have to be relatively large to produce this significant result. This provides support for the mechanism of increased below-ground investment in response to low rainfall, however, there are several other mechanisms (e.g. plant physiological, life history, successional status) which may also have contributed to above-ground drought resistance (Grime *et al.*, 2000).

Resistance to rainfall manipulation persisted for two years but, in the third experimental year, above-ground plant biomass declined considerably (- 28 %) in the rainfall manipulated plots likely driven by reductions in summer rainfall. There is insufficient data to establish whether this response was driven by the cumulative effect of three years of the rainfall manipulation treatment or alternatively the particularly high summer rainfall received at the site in year three. Continuation of the treatments into the future would be required to disentangle these two explanations. Nevertheless this finding has important implications for valuable ecosystem services (e.g. C-sequestration and livestock forage production) in the future if biomass declines in response to predicted reductions in summer rainfall are common across grasslands (Sheaffer *et al.*, 1992, Knapp & Smith, 2001).

Plant biomass declines in response to long-term reductions in rainfall have been frequently observed (Tilman & Haddi, 1992, Laporte *et al.*, 2002, Morecroft *et al.*, 2004, Harper *et al.*, 2005, Yahdjian & Sala, 2006, Evans *et al.*, 2011), however, some grassland communities have displayed long-term resistance to rainfall reductions (e.g. Grime *et al.* 2000, Jentsch *et al.* 2011). The mechanisms by which grassland ecosystems are resistant and/or resilient to changes in rainfall are poorly understood and warrant further investigation. In this study the loamy sand soils may have amplified plant biomass responses to the rainfall manipulation since rainwater is allowed to percolate through the soil more readily, hence imparting a more severe summer drought in rainfall manipulated plots than would be observed under alternative soil conditions.

Reductions in total biomass in rainfall manipulated plots were associated with a shift in the relative abundances of different plant functional types. The decline

in the ratio of grass cover to forb cover (third year only) occurred more slowly than was observed in a limestone grassland experiencing summer drought (10% of rainfall reapplied) in unusually dry conditions (Morecroft *et al.*, 2004), and in a lowland prairie experiencing 50 % lengthening of inter-rainfall dry intervals but unaltered total rainfall volumes (Fay *et al.*, 2003). Declines in grass abundances were associated with declines in three of the dominant species, with these species (*Agrostis capillaris*, *Arrhenatherum elatius* and *Holcus mollis*) being indicators of moist soils (Hill *et al.*, 1999). There were no effects of the rainfall manipulation on individual forb species or groups of forb species indicating that availability in the local seed bank determines which forbs species proliferated as the grasses decline (Jentsch *et al.*, 2011). Other grassland ecosystems may become dominated by drought adapted C4 grasses over C3 grasses in response to reduced rainfall (Knapp & Smith, 2001). Since C3 grasses are dominant in this study system, forbs may be superior competitors in response to reduced rainfall as they are long rooted and can uptake deeper, less temporally variable soil water (Schenk & Jackson, 2002).

Whilst there were plant community responses to rainfall manipulation, there was no association between N enrichment and plant composition or biomass over the three years of the experiment, despite the increases in nitrification rates and mineralisation rates within the first year of treatments commencing. This supports results from N enrichment studies in calcareous grasslands, an ecosystem which has been demonstrated to be P-limited (Wilson *et al.*, 1995, Carroll *et al.*, 2003). Generally, however, N enrichment is associated with species-losses and biomass accumulation, with rapid N acquisition in a small number of nitrophilic species fostering competitive advantages over several

subordinate species in high N conditions (Bobbink *et al.*, 2010). The lack of any measured responses in the mesotrophic grassland presented here therefore provides an interesting comparison to grassland responses to N enrichment more commonly presented. Care should be taken when interpreting these results, however, since the low replication and spatial heterogeneity present in this study resulted in relatively low statistical power, particularly for the influence of N enrichment on above-ground biomass.

Although limitation by other nutrients was not explicitly tested, an explanation for the lack of biomass and species richness response to N is that growth was limited by other plant nutrients in the soil (van der Hoek *et al.*, 2004) or alternatively that the effect size was not sufficiently large to produce a significant result. Low P availability and high N availability in the soil has been measured at this experimental site indicating that the system may be P limited (Fry, 2011). A lack of N limitation in N rich soils at this site may therefore limit plant community responses to N enrichment (Tsialtas *et al.*, 2001), though other factors such as low diversity of the plant community (Cole *et al.*, 2008) and microbial immobilisation of N could also limit plant community responses to N enrichment (Bardgett *et al.*, 2003). Leaching of N from the soil is unlikely to be preventing N driven biomass gains since a previous study at this site has demonstrated that there is low losses of N through leaching (Fry, 2011). Although this grassland system appears relatively unresponsive to increased N inputs, care must be taken in extrapolating N resistance to other grassland types where soil nutrient availability differs and the plant species present will be adapted to different nutrient and water regimes (Critchley *et al.*, 2002).

Reduction in biomass production in response to the rainfall manipulation was not prevented by N addition when both treatments were applied to plots.

Reduced rainfall and consequent low soil moisture availability can inhibit the uptake of N by plant roots, since there is less soil solution to provide N to the rhizosphere (Fay *et al.*, 2003, Lee *et al.*, 2010). This interaction may have been inhibited because on an annual basis the weather was dry during the study period meaning that even the ambient plots received low levels of rainfall, perhaps resulting in low plant N uptake (in 2010, 633 mm of annual rainfall was 17 % lower than the mean from the previous four years). P-limitation of the study system and/or the short term nature of the study may also prevent an interaction between climate and N (van der Hoek *et al.*, 2004). Despite these caveats, above-ground biomass and plant compositional responses to rainfall manipulation were not modified by N over three years in this study system.

Decreases in plant feeding *Auchenorrhyncha* in rainfall manipulated plots were likely to be due to reduced plant biomass and the loss of cell turgor pressure, inhibiting feeding under summer drought conditions (Huberty & Denno, 2004).

An association between shifts in the arthropod community and rainfall manipulated plots is in line with the findings of Boyer *et al.*, (2003), which demonstrated that changes to plant productivity were linked with arthropod community shifts in water and N manipulated grasslands. Whilst there were no effects of N, the decline in the abundance of *Auchenorrhyncha* and *Aranae* in rainfall manipulated plots suggests that the availability of prey and/or the decline in plant biomass (hunting/shelter availability) may be important drivers of carnivore abundances. Increases in *Hymenoptera* (predominantly parasites) in N addition plots could be via accelerated flowering in forbs (Burkle & Irwin,

2010), and increased availability of nectar and pollen, on which many *Hymenoptera* feed (Tooker & Hanks, 2000). A lack of drought tolerance in native herbivorous arthropod species may be beneficial for the preservation of plant productivity and C-stocks. However, future changes to rainfall regime may open up new niches for invading non-native arthropod species or native species which can feed on water-limited plants with potentially important implications for ecosystem functioning (Ward & Masters, 2007).

A multi-global change driver experiment (N, CO₂, rainfall and warming) identified N as the key driver of productivity increases in an experimental grassland (Dukes *et al.*, 2005). Another multi-driver study (N, CO₂, rainfall and warming) identified CO₂ and N as drivers of plant diversity declines (Zavaleta *et al.*, 2003). Both these findings are contrary to the results presented here, however, rainfall was increased rather than decreased and study sites were dominated by plant species adapted to a Mediterranean climate. Since future climatic changes are projected to be heterogeneous across the world (IPCC, 2008), global change manipulation experiments should seek to complement and inform global change models by simulating multiple global change factors according to predictions of how they may vary locally. This would allow the most accurate possible predictions of the effect of global change on ecosystem services. This study contributes to this knowledge gap by providing evidence that simulation of predicted changes to the timing and extent of rainfall events changed the structure and function of the grassland ecosystem, with a general reduction in ecosystem services provided. N enrichment had only minor effects and did not ameliorate or reduce climate driven plant responses.

4.6. Conclusions

This study provides evidence of short-term (< two years) resistance to rainfall manipulation (+ 15 % winter rainfall and – 30 % summer rainfall) and longer-term (> two years) resistance to N enrichment in a successional grassland ecosystem. Resistance to rainfall manipulation was short-lived, and rainfall manipulation decreased above-ground biomass in the longer-term, with plant responses not prevented by N enrichment. This has important implications for food provision, C-stocks and the climate regulating services that grasslands currently provide. Rainfall manipulation was also associated with a shift in plant and arthropod functional diversity, favouring forbs over grasses, with summer drought likely to be the key driver of change. Plant feeding and carnivorous arthropods declined in rainfall manipulated plots, hence herbivory did not add to the decline in plant productivity under rainfall manipulated conditions. The lack of resistance to drought in some native arthropods and plants indicates that invading non-native species and native disturbance-tolerant species may benefit from increased drought frequency. With future environmental changes likely, this study adds to the body of research predicting long-term shifts in the structure and function of grasslands. However, in the short-term some grassland ecosystems may be resistant to global change.

5. General discussion

5.1. Overview of key findings

This thesis aimed to provide novel and timely research adding to our understanding of the effects of air pollution and climate change in grassland ecosystems, focussing on both local and global environmental change. Initially, measured and modelled data were used to quantify road derived changes to a suite of atmospheric, edaphic, hydrological and plant variables at three calcareous grasslands (chapter 2). N enrichment was associated with increased abundances of N tolerant plant species at two of the three sites, whilst high soil moisture at the roadside was linked with increased abundance of grasses at the remaining site. These results were used to focus a second study using a smaller number of key response variables across multiple (eight) calcareous grassland sites (chapter 3). This allowed assessment of the ubiquity of the relationships identified in chapter 2. Plant species diversity and forb abundance increases at roadsides were associated with species typical of habitat edges and not typical calcareous species. Increases in the abundance of nitrophilic species was associated with dry oxidised N deposition.

This thesis also aimed to investigate the effects of global environmental changes in grassland ecosystems. A manipulation experiment was carried out to assess structural and functional responses in a mesotrophic grassland ecosystem to simulate end-of-century rainfall (+ 15 % winter rainfall and – 30 % summer rainfall) and N enrichment (chapter 4). Plant species composition and above-ground biomass were resistant to rainfall regime changes in the first two

summers but were resistant to N enrichment throughout the experiment. In the third treatment year, above-ground plant biomass declined in rainfall manipulated plots, coupled with a trend towards increasing forb abundance and declining grass abundance. There was also an associated shift in arthropod community composition following rainfall manipulation.

5.2. Roads

5.2.1. Edaphic and atmospheric conditions at roadsides

This thesis used two approaches to quantify the magnitude and extent of changes in atmospheric conditions at roadsides. Measured NO₂ concentrations at different distances from roadsides demonstrated increased concentrations of NO₂ with increasing road proximity. These measured concentrations showed that substantial regions within each site (table 5.1) were exposed to elevated vehicle derived NO₂ supporting the findings of other studies (Rodes & Holland, 1981, Bell & Ashenden, 1997, Glasius *et al.*, 1999, Roorda-Knappe *et al.*, 1999, Gilbert *et al.*, 2003, Cape *et al.*, 2004, Bernhardt-Romermann *et al.*, 2007, Bignal *et al.*, 2007). Modelled NH₃ concentrations were also shown to increase at roadsides but, due to rapid deposition of NH₃, concentrations were predicted to decline to background levels closer to the road edge than NO₂ (Cape *et al.*, 2004).

High roadside concentrations of nitrogenous compounds and low roadside concentrations of other pollutants may mean that the latter are not the main drivers of changes in the structure and function of nearby calcareous plant communities (Bignal *et al.*, 2004). Experimental fumigation of grassland plants with non-nitrogenous compounds demonstrates that they could also play a role

in driving plant compositional changes (for a review see chapter 1). In the future, increasing traffic volumes may increase emissions of these other pollutants with important implications for nearby ecosystems. The dispersal characteristics of other pollutant gases which are found at roadsides for example poly-aromatic hydrocarbons (PAH), carbon dioxide (CO₂) and ozone (O₃) are not well understood and should be investigated further (Wallington *et al.*, 2008).

Increases in the concentrations of Cu, Pb and Zn at roadsides were identified, with soil concentrations declining away from roads (chapters 2 and 3). Due to the high deposition velocity of Cu, Pb and Zn (Fowler *et al.*, 2004), soil HM concentrations declined more rapidly to background concentrations than NO₂ exhibiting similar profiles to NH₃ (table 5.1). Despite elevated concentrations of HMs at roadsides; metallophytic species did not increase nearer roadsides, however, elevated tissue Cu, Zn and Pb have been measured in bryophytes (Zechmeister *et al.*, 2006) and higher plants growing alongside roads elsewhere (Antanaitis *et al.*, 2007, Blagojevic *et al.*, 2009). Elevated HM concentrations in soil and plant tissues may, therefore, also be important drivers of plant compositional change at roadsides but may be masked by strong N effects.

Soil pH was also related to roadside proximity, with more alkaline soil found nearer to roadsides (chapter 2). In contrast, there was no general pattern in either soil moisture or salinity in relation to roadside proximity (chapters 2 and 3). There was, however, some evidence (chapter 2) that soil moisture may be increasing at roadsides adjacent to the most highly trafficked roads. In addition, there was some evidence of elevated salinity nearest roadsides at some sites. Multiple changes to air quality, soil biogeochemistry and hydrology may be

contributing to plant compositional changes in calcareous grasslands alongside roads particularly within the first 20 m of road edges, however, the key message remains that N is a key driver of change.

Table 5.1: Extent of change in air and soil parameters at roadsides based on data presented in chapters 2 and 3. M relates to soil moisture and n/a denotes that it is not appropriate to consider background concentrations for the stated factor.

		Measured / modelled	Increase / decrease at roadside	Distance from the road that background concentrations were met	Chapter
Air	NO ₂	Measured	Increase	> 200 m	2
	NH ₃	Modelled	Increase	20 m	2,3
Soil	pH	Measured	Increase	n/a	2,3
	M ^a	Measured	Increase	n/a	2,3
	Salt ^a	Measured	Increase	2.5 m	3
	Cu ^a	Measured	Increase	20 m	2,3
	Pb	Measured	Increase	20 m	2,3
	Zn	Measured	Increase	5 m	2,3

^a Increase only found at the individual site level

5.2.2. Modelling nitrogen dioxide concentrations away from roadsides

Many studies have defined NO₂ declines using an exponential function with published exponential decay rates varying from 0.005 m⁻¹ to 0.15 m⁻¹ depending on several factors including traffic density, prevailing wind direction and the maximum distance away from the roads at which NO₂ was measured (Cape *et al.*, 2004). Across the three calcareous grasslands (chapter 2), NO₂ declines away from roadsides were more accurately described using a logarithmic function. This function described the rapid decline in NO₂ concentrations within the first few metres of roadsides but the shapes of the curves were not heavily influenced by the maximum distance away from the road that measurements took place. Given the abundance of NO₂ gradient studies in the literature and the advantages of using logarithmic functions to describe NO₂ declines away from roadsides, further work should include a meta-analysis which would confirm the correct pattern of the relationship between roadside proximity and NO₂ concentrations.

NO₂ concentrations increased linearly with traffic density, with monthly NO₂ concentrations dependent upon monthly traffic densities across the three sites over the year (chapter 2). Previous studies have linked increasing NO₂ concentrations with increasing traffic density (Cape *et al.*, 2004) but this thesis contains the first study to define an interaction between traffic density and road proximity. This three-dimensional relationship enabled estimation of monthly mean NO₂ concentrations at different distances from roadsides using known traffic densities (DfT, 2011). The model was supported by a positive linear relationship between modelled and measured NO₂ concentrations at three further sites (chapter 3) indicating that the logarithmic shapes of the curves

representing the decline in NO₂ concentrations away from roadsides were well characterised.

The three test sites used in chapter 3 were selected to include topographical features or physical barriers which may affected the dispersal of pollutants and inhibited the accuracy of the model (Yin *et al.*, 2011). There was good agreement (+ 5 %) between modelled and measured NO₂ concentrations at the site where there were minimal barriers to dispersal between the road and the grassland. However, at the other two sites, overestimation of NO₂ concentrations indicated that pollutant deposition was diminished by trees positioned between the road and the grassland (- 8 %), and also when the site was raised above the road (- 28 %). Further work should seek to address this knowledge gap by testing the role of topographical features and physical barriers on the dispersal of road derived exhaust products. The influence of the prevailing wind, which has been found to be important in another study (Gilbert *et al.*, 2003), should also be considered.

5.2.3. Practical application – Management of calcareous grasslands

Land managers typically use grazing livestock (sheep and/or cattle) or mowing to prevent the accumulation of soil nutrients, to remove small populations of invading plant species and to inhibit successional changes in semi-natural calcareous habitats (Kahmen *et al.*, 2002). At the sites studied in this thesis, grazing was common across all sites (chapter 3) but an association between roadside proximity and plant compositional change remained. This suggests that grazing does not prevent all road-driven changes to calcareous grassland ecosystems.

One management option to ameliorate the effects of roads in roadside ecosystems is the use of buffer zones, which use sacrificial areas of land where road influences will occur and is common practice in agro-ecosystems. The use of buffer zones has been suggested as suitable for management of heathland located adjacent to busy roads (Angold, 1997). This technique has the advantage of not negatively impacting the aesthetic value of the landscape and being relatively cheap to implement. A buffer zone of 20 m may capture HMs, salt and basic chemicals suspended in run-off (Backstrom *et al.*, 2003). 20 m from roadsides NH_3 is also likely to decline to approximately background concentrations (Cape *et al.*, 2004). Whilst NO_2 will decline by approximately two-thirds of roadside concentrations adjacent to the busiest roads within a 20 m buffer zone (chapter 2), elevated concentrations will persist considerably further. This technique may, therefore, be beneficial for reducing the magnitude of roadside ecological changes but may not prevent the whole suite of ecological changes from occurring within calcareous grasslands located adjacent to busy roads.

Physical barriers could also inhibit road derived gaseous pollutants and run-off (Forman & Collinge, 1997, Backstrom *et al.*, 2003, DfT, 2008), evidence for which is also presented in chapters 2 and 3. Choosing between trees, shrubs and walls as physical barriers is not straightforward: walls may lead to a decline in the aesthetic value of the landscape; however, trees and shrubs may invade into nearby ecosystems leading to successional and compositional shifts (chapter 3). NO_2 concentrations adjacent to busy roads in China bordered by trees declined by 3 %, SO_2 declined by 5 % and particulates declined by 9 % compared with controls (Yin *et al.*, 2011), whereas soil and tissue HM

concentrations can decline by 40 – 60 % as a result of bordering trees (Wang *et al.*, 2007b). Walls can disrupt gaseous dispersal away from roads between 3 and 12 times the height of the walls themselves resulting in pollutant concentrations of between 0% and 80% roadside values (Hölscher *et al.*, 1993). However, interference of the flows of gases by walls may result in slower declines to background concentrations away from roadsides (Bowker *et al.*, 2007). If physical barriers are to be used to protect calcareous grasslands from road influences attention needs to be paid to the extent by which different physical barriers will inhibit the range of road derived environmental perturbations.

The current trend towards the use of electric cars which have no exhaust emissions is likely to have a positive effect on local air quality as well as global N emissions (Sioshansi & Denholm, 2009). Despite increased electricity usage associated with electric cars, overall CO₂ emissions are likely to be reduced (Perujo & Ciuffo, 2010). Whilst this may be beneficial for air quality, other road effects such as the deposition of heavy metals from brakes and tyres (Zechmeister *et al.*, 2005), salt from road gritting (Pedersen *et al.*, 2000), distribution of invasive species in tyre tread (Sera, 2010) and changes to hydrology from road run-off (Boivin *et al.*, 2008) mean that we are likely to continue to see localised ecological effects at roadsides. These ecological effects coupled with the continued usage of petrol and diesel vehicles means that researchers should consider methods of ameliorating or reducing the negative impacts of roads at sites of high conservation value. Vehicle usage is predicted to increase in the future (DfT, 2011) with unknown consequences for the structure and function of adjacent plant communities.

5.3. Grasslands and global change

5.3.1. Opportunities and challenges in understanding grassland resistance and resilience to climate change

Variations in the growth rates and changes to plant species composition in response to manipulation of rainfall regime and an overall decline in rainfall have been demonstrated across multiple grassland experiments (for a summary see chapter 1). This has important implications for carbon storage since grasslands contain 34 % of the world's terrestrial C and comprise 26 % of the most species-rich terrestrial eco-regions (White *et al.*, 2000). The extent by which grassland plant communities are resistant to reductions in rainfall i.e. showing no change to species composition, diversity and productivity varies considerably, hence predictions of future changes to biodiversity and C-stocks contain high degrees of uncertainty (Knapp & Smith, 2001, Trumper *et al.*, 2009).

Plant species richness, composition and above-ground biomass production were resistant to changes in rainfall regime manipulation for the first two treatment years in the mesotrophic grassland manipulation experiment (chapter 4). This finding complements other studies demonstrating resistance to two years of summer drought in a limestone grassland in the UK (Grime *et al.*, 2000) and in a short-grass steppe in Argentina (Yahdjian & Sala, 2006). Other studies have observed longer periods of resistance to summer drought; occurring for five years in a short-grass steppe in USA (Evans *et al.*, 2011) and for five years in a mesotrophic grassland in Germany (Jentsch *et al.*, 2011). However, the findings described in chapter 4 are in contrast with another study

which observed changes to plant species composition in response to one season of drought in an upland grassland in the USA (Tilman & Haddi, 1992).

Grassland manipulation experiments have also demonstrated that biomass and plant compositional responses to drought may persist several years after they start to appear (Morecroft *et al.*, 2004, Harper *et al.*, 2005, Yahdjian & Sala, 2006, Evans *et al.*, 2011). Other grasslands have, however, been shown to be resilient to such perturbations returning to pre-drought productivity and composition in subsequent years after the initial responses occurred (Grime *et al.*, 2000, Evans *et al.*, 2011). In the third year there was a reduction in plant biomass and a trend towards a shift in plant species composition in climate manipulated plots at the mesotrophic grassland (chapter 4). Continuation of the experiment described in chapter 4 would provide additional information to determine whether the mesotrophic grassland is resilient to the effects of rainfall manipulation over more ecologically relevant timescales.

Large variation in the extent by which different grasslands are resistant or resilient to drought demonstrates the difficulty associated with identifying general mechanisms to explain grassland responses to drought. Compensatory responses in more species rich grassland assemblages may be important (Jentsch *et al.*, 2011), however drought resistance in the dominant plant species was sufficient to explain drought resistance in a grassland ecosystem elsewhere (Evans *et al.*, 2011). Later stage successional communities have also been demonstrated to be more resistant to drought (Grime *et al.*, 2000), though this may be due to successional dominance of drought resistant plants. Defining the characteristics of the mesotrophic grassland described in chapter 4

and of other grasslands around the world which make them resistant or resilient to variation in rainfall regime remains a challenge.

5.4. Comparing mesotrophic and calcareous grassland responses to environmental change

The majority of the world's ecosystems are limited by the availability of N, with changes to plant productivity and species composition associated with N enrichment (LeBauer & Treseder, 2008, Bobbink *et al.*, 2010). Roads and road vehicles were associated with changes to plant species composition across calcareous grasslands, favouring N tolerant species at roadsides (chapters 2 and 3). In contrast N enrichment did not have a measureable effect on plant productivity or species composition in three years of mesotrophic grassland manipulation (chapter 4). Since many roads were built over 30 years ago (DfT, 2011), roadside communities will have been exposed to N enrichment over a long period allowing accumulation of soil nutrients and a gradual shift in plant community composition. Despite shorter-term exposure to N enrichment, the experimental mesotrophic grassland appears not to be primarily limited by N. This ecosystem provides an interesting comparison to other grassland ecosystems which generally increase productivity in response to N addition (Seastedt *et al.*, 1991, Tilman *et al.*, 2001, Bassin *et al.*, 2007). One explanation for this could be limitation by other nutrients such as phosphorus (van der Hoek *et al.*, 2004) or that soil microbes are acting as highly effective competitors with the plant community immobilising the additional N (Bardgett *et al.*, 2003).

Across the calcareous grasslands (chapters 2 and 3) increased soil moisture was associated with increases in the abundance of grasses, including increases

in the abundances of the mesotrophic grassland species *Dactylis glomerata* and *Holcus lanatus*. In the grassland manipulation experiment, the mesotrophic grasses *Agrostis capillaris*, *Arrhenatherum elatius* and *Holcus mollis* declined in plots which had experienced summer drought (chapter 4). This provides additional evidence that rapidly growing grasses are superior competitors where soil moisture availability is increased, but will decline in abundance when soil moisture is low (Suttle & Thomsen, 2007). In contrast, forbs increase in abundance in response to drought since they are deep rooted and can access deeper, less temporally variable water supplies (Grime *et al.*, 2000, Morecroft *et al.*, 2004, Yahdjian & Sala, 2006).

Studies of plant community responses to summer drought in limestone grasslands have demonstrated declines in sedges and grasses in response to reduced water availability, however species richness and plant biomass remained unaltered (Grime *et al.*, 2000). In roadside calcareous communities increased soil moisture content was associated with increased abundance of grasses (chapter 2). Whilst diverse limestone and chalk grasslands are generally associated with frequent drought events, chalk grasslands are also associated with low nutrient availability and basic soils (Critchley *et al.*, 2002, Bennie *et al.*, 2006). Limestone and chalk respond to rainfall in different ways, since limestone can dissolve in rain water releasing soil nutrients and acidify the soil whereas chalk is porous allowing water to pass through, accumulating water only above non-porous rocks. Since rainfall regime has not been experimentally manipulated in chalk grassland, we still do not know how calcareous grassland plant species composition and function may change in response to an increase in the frequency and severity of drought.

In the future an increased frequency of drought events in Europe may increase calcareous grassland biodiversity by favouring slow growing, drought adapted plant species, however, increased N enrichment and acidification could promote the growth and invasion of non-calcareous species (Critchley *et al.*, 2002, Bennie *et al.*, 2006, IPCC, 2008). The extent to which calcareous grasslands may be resistant and/or resilient to shifts in rainfall regimes and N enrichment is currently unknown. Changes in arthropod diversity and soil processes are also likely in response to a change in rainfall and nutrient availability (Wilson *et al.*, 1995, Carroll *et al.*, 2003). In more southerly calcareous grasslands in the UK or in Europe where rainfall is lower, drought may add excessive water stress resulting in reduced biodiversity. A long-term calcareous grassland manipulation experiment in which N enrichment, pH shifts and/or various rainfall regimes are imposed would add to our understanding of how different grasslands may respond to a changing climate and increased N deposition in the future.

5.5. Conclusion

Roads and road vehicles are a source of reactive N emissions and other environmental perturbations which are associated with changes to the composition and function of plant communities in adjacent calcareous grasslands. Vehicles also emit CO₂ and precursors to ozone (e.g. NO_x) contributing towards rising atmospheric pollutant concentrations and climate change. These climatic changes may also have important implications for the composition and functioning of plant communities. Currently grasslands provide a range of ecosystem services including pollination, climate regulation, food production and the maintenance of biodiversity. With these ecosystems likely to be exposed to novel combinations of increased N deposition, CO₂ concentrations and various aspects of climate change in the future, careful management is required if grasslands are to be maintained and are to continue to deliver current ecosystem services.

6. References

- ANGOLD P. G. (1997) The impact of a road upon adjacent heathland vegetation: Effects on plant species composition. *Journal of Applied Ecology*, **34**, 409-417.
- ANTANAITIS A., ANTANAITIS S., LUBYTE J., STAUGAITIS G. (2007) The impact of anthropogenic factors on the concentration of heavy metals in cereal grain. *Zemdirbyste (Agriculture)*, **94**, 49-58.
- AQEG (2004) *Air Quality Expert Group: Air Pollution in the UK*. Department for Environment, Food and Rural Affairs
- ASHENDEN T. W. (1979) Effects of long-term exposures to SO₂ and NO₂ pollution on the growth of *Dactylis glomerata* L and *Poa pratensis* L. *Environmental Pollution*, **18**, 249-258.
- ASHENDEN T. W., WILLIAMS I. A. D. (1980) Growth reductions in *Lolium multiflorum* L. and *Phleum pratense* L. as a result of SO₂ and NO₂ pollution. *Environmental Pollution Series A, Ecological and Biological*, **21**, 131-139.
- BACKSTROM M., NILSSON U., HAKANSSON K., ALLARD B., KARLSSON S. (2003) Speciation of heavy metals in road runoff and roadside total deposition. *Water Air and Soil Pollution*, **147**, 343-366.
- BARDGETT R. D., STREETER T. C., BOL R. (2003) Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology*, **84**, 1277-1287.
- BARGER N. N., D'ANTONIO C. M., GHNEIM T., BRINK K., CUEVAS E. (2002) Nutrient limitation to primary productivity in a secondary savanna in Venezuela. *Biotropica*, **34**, 493-501.

- BARONI F., BOSCAGLI A., PROTANO G., RICCOBONO F. (2000) Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area. *Environmental Pollution*, **109**, 347-352.
- BARRUTIA O., EPELDE L., GARCÍA-PLAZAOLA J. I., GARBISU C., BECERRIL J. M. (2009) Phytoextraction potential of two *Rumex acetosa* L. accessions collected from metalliferous and non-metalliferous sites: Effect of fertilization. *Chemosphere*, **74**, 259-264.
- BASSIN S., VOLK M., SUTER M., BUCHMANN N., FUHRER J. (2007) Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 years of treatment. *New Phytologist*, **175**, 523-534.
- BATES J. W. (1994) Responses of the mosses *Brachythecium rutabulum* and *Pseudoscleropodium purum* to a mineral nutrient pulse. *Functional Ecology*, **8**, 686-693.
- BELL J. N. B., HONOUR S. L., POWER S. A. (2011) Effects of vehicle exhaust emissions on urban wild plant species. *Environmental Pollution*, **159**, 1984-1990.
- BELL S., ASHENDEN T. W. (1997) Spatial and temporal variation in nitrogen dioxide pollution adjacent to rural roads. *Water Air and Soil Pollution*, **95**, 87-98.
- BENNIE J., HILL M. O., BAXTER R., HUNTLEY B. (2006) Influence of slope and aspect on long-term vegetation change in British chalk grasslands. *Journal of Ecology*, **94**, 355-368.

- BERNHARDT-ROMERMANN M., KUDERNATSCH T., FISCHER A. (2007)
Traffic born pollution affects vegetation composition of coniferous forests.
Forstarchiv, **78**, 46-54.
- BIGNAL K., POWER S., ASHMORE M. (2004) The ecological effects of diffuse
air pollution from road transport. English Nature Research Reports 580,
Peterborough, UK: English Nature.
- BIGNAL K. L., ASHMORE M. R., HEADLEY A. D., STEWART K., WEIGERT K.
(2007) Ecological impacts of air pollution from road transport on local
vegetation. *Applied Geochemistry*, **22**, 1265-1271.
- BINNIE J., CAPE J. N., MACKIE N., LEITH I. D. (2002) Exchange of organic
solvents between the atmosphere and grass - the use of open top
chambers. *Science of the Total Environment*, **285**, 53-67.
- BLAGOJEVIC N., DAMJANOVIC-VRATNICA B., VUKASINOVIC-PESIC V.,
DUROVIC D. (2009) Heavy Metals Content in Leaves and Extracts of
Wild-Growing *Salvia Officinalis* from Montenegro. *Polish Journal of
Environmental Studies*, **18**, 167-173.
- BOBBINK R., HATTELINGH J. P. (2011) Review and revision of empirical
critical loads and dose-response relationships. *Proceedings of an expert
workshop, Noordwijkerhout, 23–25 June 2010*. Co-ordination centre for
effects, National Institute for Public Health and the Environment (RIVM).
- BOBBINK R., HICKS K., GALLOWAY J., SPRANGER T., ALKEMADE R.,
ASHMORE M., BUSTAMANTE M., CINDERBY S., DAVIDSON E.,
DENTENER F., EMMETT B., ERISMAN J. W., FENN M., GILLIAM F.,
NORDIN A., PARDO L., DE VRIES W. (2010) Global assessment of

- nitrogen deposition effects on terrestrial plant diversity: a synthesis.
Ecological Applications, **20**, 30-59.
- BOBBINK R., HORNUNG M., ROELOFS J. G. M. (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, **86**, 717-738.
- BOIVIN P., SAADE M., PFEIFFER H. R., HAMMECKER C., DEGOUMOIS Y. (2008) Depuration of highway runoff water into grass-covered embankments. *Environmental Technology*, **29**, 709-720.
- BOWKER G. E., BALDAUF R., ISAKOV V., KHLYSTOV A., PETERSEN W. (2007) The effects of roadside structures on the transport and dispersion of ultrafine particles from highways. *Atmospheric Environment*, **41**, 8128-8139.
- BOYER A. G., SWEARINGEN R. E., BLAHA M. A., FORTSON C. T., GREMILLION S. K., OSBORN K. A., MORAN M. D. (2003) Seasonal variation in top-down and bottom-up processes in a grassland arthropod community. *Oecologia*, **136**, 309-316.
- BROOK A. J., WOODCOCK B. A., SINKA M., VANBERGEN A. J. (2008) Experimental verification of suction sampler capture efficiency in grasslands of differing vegetation height and structure. *Journal of Applied Ecology*, **45**, 1357-1363.
- BROWN G., BRINKMANN K. (1992) Heavy metal tolerance in *Festuca ovina* L. from contaminated sites in the Eifel Mountains, Germany. *Plant and Soil*, **143**, 239-247.

- BURKLE L., IRWIN R. (2010) Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. *Journal of Ecology*, **98**, 705-717.
- CAKMAK I., OZTURK L., EKER S., TORUN B., KALFA H. I., YILMAZ A. (1997) Concentration of zinc and activity of copper/zinc-superoxide dismutase in leaves of rye and wheat cultivars differing in sensitivity to zinc deficiency. *Journal of Plant Physiology*, **151**, 91-95.
- CAPE J. N. (2009) The use of passive diffusion tubes for measuring concentrations of nitrogen dioxide in air. *Critical Reviews in Analytical Chemistry*, **39**, 289-310.
- CAPE J. N., TANG Y. S., VAN DIJK N., LOVE L., SUTTON M. A., PALMER S. C. F. (2004) Concentrations of ammonia and nitrogen dioxide at roadside verges, and their contribution to nitrogen deposition. *Environmental Pollution*, **132**, 469-478.
- CAPE J. N., VAN DER EERDEN L. J., SHEPPARD L. J., LEITH I. D., SUTTON M. A. (2009) Evidence for changing the critical level for ammonia. *Environmental Pollution*, **157**, 1033-1037.
- CARROLL J. A., CAPORN S. J. M., JOHNSON D., MORECROFT M. D., LEE J. A. (2003) The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution*, **121**, 363-376.
- CARSLAW D. C., BEEVERS S. D. (2005) Estimations of road vehicle primary NO₂ exhaust emission fractions using monitoring data in London. *Atmospheric Environment*, **39**, 167-177.

- CARSLAW D. C., BEEVERS S. D., WESTMORELAND E., WILLIAMS M. L.,
TATE J. E., MURRELLS T., STEDMAN J. L., Y., GRICE S., KENT A.,
TSAGATAKIS I. (2011) *Trends in NO_x and NO₂ emissions and ambient measurements in the UK*. Department for Environment, Food, and Rural Affairs.
- CLARK C. M., CLELAND E. E., COLLINS S. L., FARGIONE J. E., GOUGH L.,
GROSS K. L., PENNINGS S. C., SUDING K. N., GRACE J. B. (2007)
Environmental and plant community determinants of species loss
following nitrogen enrichment. *Ecology Letters*, **10**, 596-607.
- COLE L., BUCKLAND S. M., BARDGETT R. D. (2008) Influence of disturbance
and nitrogen addition on plant and soil animal diversity in grassland. *Soil Biology & Biochemistry*, **40**, 505-514.
- COUNTRYSIDE-SURVEY (2008) *Countryside Survey: UK results from 2007*.
Centre for Ecology and Hydrology.
- CRAWLEY M. J. (2007) *The R book*. Oxford, UK: Wiley.
- CRITCHLEY C. N. R., CHAMBERS B. J., FOWBERT J. A., SANDERSON R. A.,
BHOGAL A., ROSE S. C. (2002) Association between lowland grassland
plant communities and soil properties. *Biological Conservation*, **105**, 199-
215.
- DANIEL P., KOVACS B., PROKISCH J., GYORI Z. (1997) Heavy metal
dispersion detected in soils and plants alongside roads in Hungary.
Chemical Speciation and Bioavailability, **9**, 83-93.
- DEFRA (2007) *The Air Quality Strategy for England, Scotland, Wales and Northern Ireland (Volume 1)*. Department for Environment, Food and Rural Affairs.

DEFRA (2011) *UK-Air*. [Online]. Available from: <http://uk-air.defra.gov.uk>

[Accessed 16 August 2011].

DENTENER F., DREVET J., LAMARQUE J. F., BEY I., EICKHOUT B., FIORE A. M., HAUGLUSTAINE D., HOROWITZ L. W., KROL M., KULSHRESTHA U. C., LAWRENCE M., GALY-LACAUX C., RAST S., SHINDELL D., STEVENSON D., NOIJE T. V., ATHERTON C., BELL N., BERGMAN D., BUTLER T., COFALA J., COLLINS B., DOHERTY R., ELLINGSEN K., GALLOWAY J., GAUSS M. (2006) Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation. *Global Biogeochemical Cycles*, **20**.

DERAM A., LANGUEREAU-LEMAN F., HOWSAM M., PETIT D., HALUWYN C. V. (2008) Seasonal patterns of cadmium accumulation in *Arrhenatherum elatius* (Poaceae): Influence of mycorrhizal and endophytic fungal colonisation. *Soil Biology and Biochemistry*, **40**, 845-848.

DFT (2008) *Alternative filter media and surface stabilisation techniques for combined surface and sub-surface drain*. Department for Transport.

DFT (2011) *Transport statistics*. [Online]. Available from:

<http://www.dft.gov.uk/statistics> [Accessed 1 September 2011].

DUECK T. A., VANDEREERDEN L. J., BEEMSTERBOER B., ELDERSON J. (1991) Nitrogen uptake and allocation by *Calluna vulgaris* (L) HULL and *Deschampsia flexuosa* (L) TRIN exposed to $^{15}\text{NH}_3$. *Acta Botanica Neerlandica*, **40**, 257-267.

DUKES J. S., CHIARIELLO N. R., CLELAND E. E., MOORE L. A., SHAW M. R., THAYER S., TOBECK T., MOONEY H. A., FIELD C. B. (2005)

- Responses of grassland production to single and multiple global environmental changes. *Plos Biology*, **3**, 1829-1837.
- EEA (2010) *The European Environment State and Outlook 2010: Biodiversity*. European Environment Agency.
- EISENHAUER N., CESARZ S., KOLLER R., WORM K., REICH P. B. (2011) Global change below ground: impacts of elevated CO₂, nitrogen and summer drought on soil food webs and biodiversity. *Global Change Biology*, **18**, 435-447.
- ERF (2010) *European Road Statistics 2010*. European Union - Road Federation.
- ERISMAN J. W., SUTTON M. A., GALLOWAY J., KLIMONT Z., WINIWARTER W. (2008) How a century of ammonia synthesis changed the world. *Nature Geoscience*, **1**, 636-639.
- EVANS S. E., BYRNE K. M., LAUENROTH W. K., BURKE I. C. (2011) Defining the limit to resistance in a drought-tolerant grassland: long-term severe drought significantly reduces the dominant species and increases ruderals. *Journal of Ecology*, **99**, 1500-1507.
- FAY P. A., CARLISLE J. D., KNAPP A. K., BLAIR J. M., COLLINS S. L. (2003) Productivity responses to altered rainfall patterns in a C4-dominated grassland. *Oecologia*, **137**, 245-251.
- FIALA K., TŮMA I., HOLUB P. (2009) Effect of manipulated rainfall on root production and plant belowground dry mass of different grassland ecosystems. *Ecosystems*, **12**, 906-914.
- FISCHER M., STOCKLIN J. (1997) Local extinctions of plants in remnants of extensively used calcareous grasslands 1950-1985. *Conservation Biology*, **11**, 727-737.

- FORMAN R. T. T., COLLINGE S. K. (1997) Nature conserved in changing landscapes with and without spatial planning. *Landscape and Urban Planning*, **37**, 129-135.
- FOWLER D., SKIBA U., NEMITZ E., CHOUBEDAR F., BRANFORD D., DONOVAN R., ROWLAND P. (2004) Measuring aerosol and heavy metal deposition on urban woodland and grass using inventories of Pb and metal concentrations in soil. *Water, Air, & Soil Pollution*, **4**, 483-499.
- FRASER L. H., GRIME J. P. (1998) Top-down control and its effect on the biomass and composition of three grasses at high and low soil fertility in outdoor microcosms. *Oecologia*, **113**, 239-246.
- FRY E. (2011) Climate change implications for grassland ecosystems: a biodiversity approach. *PhD thesis (Imperial College London)*.
- GALLOWAY J. N., DENTENER F. J., CAPONE D. G., BOYER E. W., HOWARTH R. W., SEITZINGER S. P., ASNER G. P., CLEVELAND C. C., GREEN P. A., HOLLAND E. A., KARL D. M., MICHAELS A. F., PORTER J. H., TOWNSEND A. R., VOROSMARTY C. J. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153-226.
- GALLOWAY J. N., TOWNSEND A. R., ERISMAN J. W., BEKUNDA M., CAI Z. C., FRENEY J. R., MARTINELLI L. A., SEITZINGER S. P., SUTTON M. A. (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science*, **320**, 889-892.
- GELBARD J. L., BELNAP J. (2003) Roads as conduits for exotic plant invasions in a semiarid landscape. *Conservation Biology*, **17**, 420-432.

- GILBERT N. L., WOODHOUSE S., STIEB D. M., BROOK J. R. (2003) Ambient nitrogen dioxide and distance from a major highway. *Science of the Total Environment*, **312**, 43-46.
- GLASIUS M., CARLSEN M. F., HANSEN T. S., LOHSE C. (1999) Measurements of nitrogen dioxide on Funen using diffusion tubes. *Atmospheric Environment*, **33**, 1177-1185.
- GOUGH L., RAMSEY E. A., JOHNSON D. R. (2007) Plant-herbivore interactions in Alaskan arctic tundra change with soil nutrient availability. *Oikos*, **116**, 407-418.
- GRAHAM R. D. (1981) Advances in research on copper deficiency in cereals. *Australian Journal of Agricultural Research*, **32**, 1009-1037.
- GREEN S. M., CRESSER M. S. (2008) Nitrogen cycle disruption through the application of de-icing salts on upland highways. *Water Air and Soil Pollution*, **188**, 139-153.
- GRIME J. P., BROWN V. K., THOMPSON K., MASTERS G. J., HILLIER S. H., CLARKE I. P., ASKEW A. P., CORKER D., KIELTY J. P. (2000) The response of two contrasting limestone grasslands to simulated climate change. *Science*, **289**, 762-765.
- HANSON P. J., LINDBERG S. E. (1991) Dry deposition of reactive nitrogen compounds - A review of leaf, canopy and non-foliar measurements. *Atmospheric Environment*, **25**, 1615-1634.
- HARPER C. W., BLAIR J. M., FAY P. A., KNAPP A. K., CARLISLE J. D. (2005) Increased rainfall variability and reduced rainfall amount decreases soil CO₂ flux in a grassland ecosystem. *Global Change Biology*, **11**, 322-334.

- HARPOLE W. S., POTTS D. L., SUDING K. N. (2007) Ecosystem responses to water and nitrogen amendment in a California grassland. *Global Change Biology*, **13**, 2341-2348.
- HARRISON R. M., JOHNSTON W. R. (1985) Deposition fluxes of lead, cadmium, copper and polynuclear aromatic hydrocarbons (PAH) on the verges of a major highway. *Science of the Total Environment*, **46**, 121-135.
- HARRISON R. M., PEAK J. D., COLLINS G. M. (1996) Tropospheric cycle of nitrous acid. *Journal of Geophysical Research*, **101**, 14429-14439.
- HILL M. O., MOUNTFORD J. O., ROY D. B., BUNCE R. G. H. (1999) *Ellenberg's indicator values for British plants. ECOFACT Volume 2: technical annex*. Centre for Ecology and Hydrology.
- HILL M. O., PRESTON C. D., ROY D. B. (2004) *PLANTATT - Attributes of British and Irish plants: status, size, life history, geography and habitats*. Centre for Ecology & Hydrology.
- HODGSON J. G., GRIME J. P., WILSON P. J., THOMPSON K., BAND S. R. (2005) The impacts of agricultural change (1963-2003) on the grassland flora of Central England: processes and prospects. *Basic and Applied Ecology*, **6**, 107-118.
- HÖLSCHER N., HÖFFER R., NIEMANN H.-J., BRILON W., ROMBERG E. (1993) Wind tunnel experiments on micro-scale dispersion of exhausts from motorways. *Science of the Total Environment*, **134**, 71-79.
- HONOUR S. L., BELL J. N. B., ASHENDEN T. W., CAPE J. N., POWER S. A. (2009) Responses of herbaceous plants to urban air pollution: effects on

- growth, phenology and leaf surface characteristics. *Environmental Pollution*, **157**, 1279-1286.
- HOOPER D. U., JOHNSON L. (1999) Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation. *Biogeochemistry*, **46**, 247-293.
- HUBERTY A. F., DENNO R. F. (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, **85**, 1383-1398.
- IPCC (2007) Summary for policymakers. In *Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change* (eds Solomon, S. Qin, D. Manning, Z. Chen, M. Marquis, K. B. Avery, M. Tignor & H.L. Miller), pp 12 - 18. Cambridge, UK: Cambridge University Press.
- IPCC (2008) *Climate change and water*. Technical Paper of the Intergovernmental Panel on Climate Change. Geneva, Switzerland: Intergovernmental Panel on Climate Change.
- JABER F. H., SHUKLA S., STOFFELLA P. J., OBREZA T. A., HANLON E. A. (2005) Impact of organic amendments on groundwater nitrogen concentrations for sandy and calcareous soils. *Compost Science & Utilization*, **13**, 194-202.
- JENTSCH A., KREYLING J., ELMER M., GELLESCH E., GLASER B., GRANT K., HEIN R., LARA M., MIRZAE H., NADLER S. E., NAGY L., OTIENO D., PRITSCH K., RASCHER U., SCHÄDLER M., SCHLOTER M., SINGH B. K., STADLER J., WALTER J., WELLSTEIN C., WÖLLECKE J., BEIERKUHNLEIN C. (2011) Climate extremes initiate ecosystem-

- regulating functions while maintaining productivity. *Journal of Ecology*, **99**, 689-702.
- KAHMEN S., POSCHLOD P., SCHREIBER K.-F. (2002) Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. *Biological Conservation*, **104**, 319-328.
- KALAPOUS T., VAN DEN BOOGAARD R., LAMBERS H. (1996) Effect of soil drying on growth, biomass allocation and leaf gas exchange of two annual grass species. *Plant and Soil*, **185**, 137-149.
- KAMMERBAUER J., DICK T. (2000) Monitoring of urban traffic emissions using some physiological indicators in *Ricinus communis* L. plants. *Archives of Environmental Contamination and Toxicology*, **39**, 161-166.
- KING P. M. (1974) Copper deficiency symptoms in wheat. *Journal of Agriculture*, **77**, 96-99.
- KLEIN GOLDEWIJK K., BEUSEN A., VAN DRECHT G., DE VOS M. (2011) The HYDE 3.1 spatially explicit database of human-induced global land-use change over the past 12,000 years. *Global Ecology and Biogeography*, **20**, 73-86.
- KNAPP A. K., SMITH M. D. (2001) Variation among biomes in temporal dynamics of aboveground primary production. *Science*, **291**, 481-484.
- KROMMER V., ZECHMEISTER H. G., RÖDER I., SCHARF S., HANUS-ILLNAR A. (2007) Monitoring atmospheric pollutants in the biosphere reserve Wienerwald by a combined approach of biomonitoring methods and technical measurements. *Chemosphere*, **67**, 1956-1966.

- LAFFRAY X., ROSE C., GARREC J. P. (2010) Biomonitoring of traffic-related nitrogen oxides in the Maurienne valley (Savoie, France), using purple moor grass growth parameters and leaf N-15/N-14 ratio. *Environmental Pollution*, **158**, 1652-1660.
- LAPORTE M., DUCHESNE L., WETZEL S. (2002) Effect of rainfall patterns on soil surface CO₂ efflux, soil moisture, soil temperature and plant growth in a grassland ecosystem of northern Ontario, Canada: implications for climate change. *BMC Ecology*, **2**, 10.
- LASCHNER C., LIMBECK A., RENDL J., PUXBAUM H. (2004) Particulate emissions from on-road vehicles in the Kaisermuhlen-tunnel (Vienna, Austria). *Atmospheric Environment*, **38**, 2187-2195.
- LAUENROTH W. K., DODD J. L., SIMS P. L. (1978) The effects of water- and nitrogen-induced stresses on plant community structure in a semiarid grassland. *Oecologia*, **36**, 211-222.
- LEBAUER D. S., TRESEDER K. K. (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, **89**, 371-379.
- LEE M., DAVIES L., POWER S. (2012) Effects of roads on adjacent plant community composition and ecosystem function: an example from three calcareous ecosystems. *Environmental Pollution*, **163**, 273-280.
- LEE M., MANNING P., RIST J., POWER S. A., MARSH C. (2010) A global comparison of grassland biomass responses to CO₂ and nitrogen enrichment. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 2047-2056.

- LI F., KANG L., GAO X., HUA W., YANG F., HEI W. (2007) Traffic-related heavy metal accumulation in soils and plants in Northwest China. *Soil & Sediment Contamination*, **16**, 473-484.
- LITTLE P., WIFFEN R. D. (1978) Emission and deposition of lead from motor exhausts — II. Airborne concentration, particle size and deposition of lead near motorways. *Atmospheric Environment* (1967), **12**, 1331-1341.
- LUCE C. H., CUNDY T. W. (1994) Parameter identification for a runoff model for forest roads. *Water Resources Research*, **30**, 1057-1069.
- MARSH D. M., BECKMAN N. G. (2004) Effects of forest roads on the abundance and activity of terrestrial salamanders. *Ecological Applications*, **14**, 1882-1891.
- MARTÍNEZ C. E., MOTTO H. L. (2000) Solubility of lead, zinc and copper added to mineral soils. *Environmental Pollution*, **107**, 153-158.
- MATTSON W. J., HAACK R. A. (1987) The role of drought in outbreaks of plant eating insects. *Bioscience*, **37**, 110-118.
- MEA (2006) *Ecosystems and human well being: synthesis report*. Millennium Ecosystem Assessment. Washington DC, USA: Island Press.
- METOFFICE (2011) *UK Climate*. [Online]. Available from: <http://www.metoffice.gov.uk/climate/uk> [Accessed 26 November 2011].
- MOOG D., POSCHLOD P., KAHMEN S., SCHREIBER K. F. (2002) Comparison of species composition between different grassland management treatments after 25 years. *Applied Vegetation Science*, **5**, 99-106.
- MORECROFT M. D., MASTERS G. J., BROWN V. K., CLARKE I. P., TAYLOR M. E., WHITEHOUSE A. T. (2004) Changing precipitation patterns alter

- plant community dynamics and succession in an ex-arable grassland.
Functional Ecology, **18**, 648-655.
- MORTIMER S. R., HOLLIER J. A., BROWN V. K. (1998) Interactions between plant and insect diversity in the restoration of lowland calcareous grasslands in southern Britain. *Applied Vegetation Science*, **1**, 101-114.
- MURPHY J. M., SEXTON D. M. H., JENKINS G. J., BOORMAN P. M., BOOTH B. B. B., BROWN C. C., CLARK R. T., COLLINS M., HARRIS G. R., KENDON E. J., BETTS R. J., BROWN S. J., HOWARD T. P., HUMPREY K. A., MCCARTHEY M. P., MCDONALD R. E., STEPHENS A., WALLACE C., WARREN R., WILBY R., WOOD R. A. (2009) *UK Climate Projections Science Report: Climate change projections*. Met Office Hadley Centre, Exeter.
- PAGOTTO C., REMY N., LEGRET M., LE CLOIREC P. (2001) Heavy metal pollution of road dust and roadside soil near a major rural highway. *Environmental Technology*, **22**, 307-319.
- PALMES E. D., GUNNISON A. F., DIMATTIO J., TOMCZYK C. (1976) Personal sampler for nitrogen-dioxide. *American Industrial Hygiene Association Journal*, **37**, 570-577.
- PEDERSEN L. B., RANDRUP T. B., INGERSLEV M. (2000) Effects of road distance and protective measures on deicing NaCl deposition and soil solution chemistry in planted median strips. *Journal of Arboriculture*, **26**, 238-245.
- PEDRO SILVA J., TOLAND J., JONES W., ELDRIDGE J., THORPE E., O'HARA E. (2008) *LIFE and Europe's grasslands - Restoring a forgotten habitat*. Available at <http://ec.europa.eu>. European Union.

- PERUJO A., CIUFFO B. (2010) The introduction of electric vehicles in the private fleet: Potential impact on the electric supply system and on the environment. A case study for the Province of Milan, Italy. *Energy Policy*, **38**, 4549-4561.
- PHOENIX G. K., BOOTH R. E., LEAKE J. R., READ D. J., GRIME J. P., LEE J. A. (2003) Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands. *Global Change Biology*, **9**, 1309-1321.
- PILON M., ABDEL-GHANY S. E., COHU C. M., GOGOLIN K. A., YE H. (2006) Copper cofactor delivery in plant cells. *Current Opinion in Plant Biology*, **9**, 256-263.
- PINHIERO J. C., BATES D. M. (2001) *Mixed-effects models in S and S-PLUS*. New York, USA: Springer.
- PITCAIRN C., FOWLER D., LEITH I., SHEPPARD L., TANG S., SUTTON M., FAMULARI D. (2006) Diagnostic indicators of elevated nitrogen deposition. *Environmental Pollution*, **144**, 941-950.
- PITCAIRN C. E. R., FOWLER D., LEITH I. D., SHEPPARD L. J., SUTTON M. A., KENNEDY V., OKELLO E. (2003) Bioindicators of enhanced nitrogen deposition. *Environmental Pollution*, **126**, 353-361.
- PLEIJEL H., DANIELSSON H. (1997) Growth of 27 herbs and grasses in relation to ozone exposure and plant strategy. *New Phytologist*, **135**, 361-367.
- RADIN J. W., MAUNEY J. R., GUINN G. (1985) Effects of N-fertility on plant water relations and stomatal responses to water-stress in irrigated cotton. *Crop Science*, **25**, 110-115.

- RAGAB R., ROSIER P., DIXON A., BROMLEY J., COOPER J. D. (2003)
Experimental study of water fluxes in a residential area: 2. Road infiltration, runoff and evaporation. *Hydrological Processes*, **17**, 2423-2437.
- REIDSMA P., TEKELENBURG T., VAN DEN BERG M., ALKEMADE R. (2006)
Impacts of land-use change on biodiversity: An assessment of agricultural biodiversity in the European Union. *Agriculture, Ecosystems & Environment*, **114**, 86-102.
- RODES C. E., HOLLAND D. M. (1981) Variations of NO, NO₂ and O₃ concentrations downwind of a Los Angeles freeway. *Atmospheric Environment*, **15**, 243-250.
- RODWELL (1992) *British plant communities. Volume 3. Grasslands and montane communities*. Cambridge, UK: Cambridge University Press.
- ROORDA-KNAPE M. C., JANSSEN N. A. H., DE HARTOG J., VAN VLIET P. H. N., HARSSEMA H., BRUNEKREEF B. (1999) Traffic related air pollution in city districts near motorways. *Science of the Total Environment*, **235**, 339-341.
- ROSS S. M., KAYE K. J. (1994) The meaning of metal toxicity in soil-plant systems. In: *Toxic metals in soil-plant systems*. (ed Ross SM) pp 27-61. Oxford, UK: Wiley.
- ROTAP (2011) Review of Transboundary Air Pollution (RoTAP), A review of Acidification, Eutrophication, Heavy Metals and Ground-Level Ozone in the UK. Available at www.rotap.ceh.ac.uk. Centre for Ecology and Hydrology.

- ROWLAND A. J., DREW M. C., WELLBURN A. R. (1987) Foliar entry and incorporation of atmospheric nitrogen dioxide into barley plants of different nitrogen status. *New Phytologist*, **107**, 357-371.
- SALA O. E., CHAPIN F. S., ARMESTO J. J., BERLOW E., BLOOMFIELD J., DIRZO R., HUBER-SANWALD E., HUENNEKE L. F., JACKSON R. B., KINZIG A., LEEMANS R., LODGE D. M., MOONEY H. A., OESTERHELD M., POFF N. L., SYKES M. T., WALKER B. H., WALKER M., WALL D. H. (2000) Biodiversity - Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770-1774.
- SCHENK H. J., JACKSON R. B. (2002) Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. *Journal of Ecology*, **90**, 480-494.
- SCOTT N. E., DAVISON A. W. (1985) The distribution and ecology of coastal species on roadsides. *Vegetatio*, **62**, 433-440.
- SCURLOCK J. M. O., HALL D. O. (1998) The global carbon sink: a grassland perspective. *Global Change Biology*, **4**, 229-233.
- SEASTEDT T. R., BRIGGS J. M., GIBSON D. J. (1991) Controls of Nitrogen Limitation in Tallgrass Prairie. *Oecologia*, **87**, 72-79.
- SEHMEL G. A. (1980) Particle and gas dry deposition: A review. *Atmospheric Environment*, **14**, 983-1011.
- SERA B. (2010) Road-Side Herbaceous Vegetation: Life History Groups and Habitat Preferences. *Polish Journal of Ecology*, **58**, 69-79.
- SHANGGUAN Z. P., SHAO M. A., DYCKMANS J. (2000) Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use

- efficiency in winter wheat. *Environmental and Experimental Botany*, **44**, 141-149.
- SHEAFFER C. C., PETERSON P. R., HALL M. H., STORDAHL J. B. (1992) Drought effects on yield and quality of perennial grasses in the north central United States. *Journal of Production Agriculture*, **4**, 556-561.
- SHEPPARD L. J., LEITH I. D., CROSSLEY A., VAN DIJK N., CAPE J. N., FOWLER D., SUTTON M. A. (2009) Long-term cumulative exposure exacerbates the effects of atmospheric ammonia on an ombrotrophic bog: Implications for critical levels. In: *Atmospheric Ammonia: Detecting emission changes and environmental impacts*. (eds Sutton MA, Reis S, Baker SMH) pp 49-58. London, UK: Springer.
- SHEPPARD L. J., LEITH I. D., MIZUNUMA T., NEIL CAPE J., CROSSLEY A., LEESON S., SUTTON M. A., VAN DIJK N., FOWLER D. (2011) Dry deposition of ammonia gas drives species change faster than wet deposition of ammonium ions: evidence from a long-term field manipulation. *Global Change Biology*, **17**, 3589-3607.
- SIOHANSI R., DENHOLM P. (2009) Emissions Impacts and Benefits of Plug-In Hybrid Electric Vehicles and Vehicle-to-Grid Services. *Environmental Science & Technology*, **43**, 1199-1204.
- SPELLERBERG I. A. N. (1998) Ecological effects of roads and traffic: a literature review. *Global Ecology and Biogeography*, **7**, 317-333.
- STEVENS C. J., DISE N. B., GOWING D. J. G., MOUNTFORD J. O. (2006) Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. *Global Change Biology*, **12**, 1823-1833.

- STEVENS C. J., DISE N. B., MOUNTFORD J. O., GOWING D. J. (2004)
Impact of nitrogen deposition on the species richness of grasslands.
Science, **303**, 1876-1879.
- STEVENS C. J., MANNING P., VAN DEN BERG L. J. L., DE GRAAF M. C. C.,
WAMELINK G. W. W., BOXMAN A. W., BLEEKER A., VERGEER P.,
ARRONIZ-CRESPO M., LIMPENS J., LAMERS L. P. M., BOBBINK R.,
DORLAND E. (2011) Ecosystem responses to reduced and oxidised
nitrogen inputs in European terrestrial habitats. *Environmental Pollution*,
159, 665-676.
- SU Y. S., WANIA F., HARNER T., LEI Y. D. (2007) Deposition of
polybrominated diphenyl ethers, polychlorinated biphenyls, and
polycyclic aromatic hydrocarbons to a boreal deciduous forest.
Environmental Science & Technology, **41**, 534-540.
- SUTTIE J. M., REYNOLDS S. G., BATELLO C. (2005) *Grasslands of the world*.
Rome, Italy: Food and Agricultural Organisation of the United Nations.
Plant Production and Protection Series No. 34.
- SUTTLE K. B., THOMSEN M. A. (2007) Climate change and grassland
restoration in California: Lessons from six years of rainfall manipulation in
a north coast grassland. *Madrone*, **54**, 225-233.
- SUTTLE K. B., THOMSEN M. A., POWER M. E. (2007) Species interactions
reverse grassland responses to changing climate. *Science*, **315**, 640-642.
- TAGUE C., BAND L. (2001) Simulating the impact of road construction and
forest harvesting on hydrologic response. *Earth Surface Processes and
Landforms*, **26**, 135-151.

- TESHA A. J., KUMAR D. (1978) Effects of fertilizer nitrogen on drought resistance in *Coffea arabica* L. *Journal of Agricultural Science*, **90**, 625-631.
- THOMPSON J. R., RUTTER A. J., RIDOUT P. S. (1986) The salinity of motorway soils. II. Distance from the carriageway and other sources of local variation in salinity. *Journal of Applied Ecology*, **23**, 269-280.
- TILMAN D., HADDI A. (1992) Drought and biodiversity in Grasslands. *Oecologia*, **89**, 257-264.
- TILMAN D., REICH P. B., KNOPS J., WEDIN D., MIELKE T., LEHMAN C. (2001) Diversity and productivity in a long-term grassland experiment. *Science*, **294**, 843-845.
- TOBAR R. M., AZCÓN R., BAREA J. M. (1994) The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. *Mycorrhiza*, **4**, 105-108.
- TOOKER J. F., HANKS L. M. (2000) Flowering plant hosts of adult Hymenopteran parasitoids of central Illinois. *Annals of the Entomological Society of America*, **93**, 580-588.
- TRUMPER K., BERTZKY M., DICKSON B., VAN DER HEIJDEN G., JENKINS M., MANNING P. (2009) *The natural fix? The role of ecosystems in climate mitigation*. Cambridge, UK: UNEP-WCMC. UNEP Rapid Response Assessment. United Nations Environment Programme.
- TRUSCOTT A. M., PALMER S. C. F., MCGOWAN G. M., CAPE J. N., SMART S. (2005) Vegetation composition of roadside verges in Scotland: the effects of nitrogen deposition, disturbance and management. *Environmental Pollution*, **136**, 109-118.

- TSIALTAS J. T., HANDLEY L. L., KASSIOUMI M. T., VERESOGLOU D. S., GAGIANAS A. A. (2001) Interspecific variation in potential water-use efficiency and its relation to plant species abundance in a water-limited grassland. *Functional Ecology*, **15**, 605-614.
- UKBAP (1994) UK Biodiversity Action Plan. (<http://www.ukbap.org.uk>). .
- UKBAP (2007) UK Biodiversity Action Plan. Report on the Species and Habitat Review (<http://www.ukbap.org.uk>).
- UVEGES J. L., CORBETT A. L., MAL T. K. (2002) Effects of lead contamination on the growth of *Lythrum salicaria* (purple loosestrife). *Environmental Pollution*, **120**, 319-323.
- VAN DEN BERG L. J. L., VERGEER P., RICH T. C. G., SMART S. M., GUEST D. A. N., ASHMORE M. R. (2011) Direct and indirect effects of nitrogen deposition on species composition change in calcareous grasslands. *Global Change Biology*, **17**, 1871-1883.
- VAN DER EERDEN L. J., DUECK T. A., BERDOWSKI J. J. M., GREVEN H., VAN DOBBEN H. F. (1991) Influence of ammonia and ammonium sulfate on heathland vegetation. *Acta Botanica Neerlandica*, **40**, 281-296.
- VAN DER HOEK D., VAN MIERLO A., VAN GROENENDAEL J. M. (2004) Nutrient limitation and nutrient-driven shifts in plant species composition in a species-rich fen meadow. *Journal of Vegetation Science*, **15**, 389-396.
- VAN DER WIEL H. J. (2003) *Determination of elements by ICP-AES and ICP-MS*. Bilthoven, Germany: National Institute of Public Health and the Environment.

- VARDAR N., ODABASI M., HOLSEN T. M. (2002) Particulate dry deposition and overall deposition velocities of polycyclic aromatic hydrocarbons. *Journal of Environmental Engineering*, **128**, 269-274.
- VIRTANEN R., JOHNSTON A. E., CRAWLEY M. J., EDWARDS G. R. (2000) Bryophyte biomass and species richness on the Park Grass Experiment, Rothamsted, UK. *Plant Ecology*, **151**, 129-141.
- VISKARI E. L., HOLOPAINEN T., KARENLAMPI L. (2000) Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions - II - ultrastructural changes and stomatal behaviour. *Environmental Pollution*, **107**, 99-107.
- VISKARI E. L., REKILA R., ROY S., LEHTO O., RUUSKANEN J., KARENLAMPI L. (1997) Airborne pollutants along a roadside: Assessment using snow analyses and moss bags. *Environmental Pollution*, **97**, 153-160.
- WALLINGTON T. J., SULLIVAN J. L., HURLEY M. D. (2008) Emissions of CO₂, CO, NO_x, HC, PM, HFC-134a, N₂O and CH₄ from the global light duty vehicle fleet. *Meteorologische Zeitschrift*, **17**, 109-116.
- WANG C., CHEN Z.-L., WANG J., ZHOU N.-S., XU S.-Y. (2007a) Heavy metal pollution of soils and vegetables on roadsides in Chongming Island. *Journal of Ecology and Rural Environment*, **23**, 89-93.
- WANG C., QIE G., YANG Y., REN Q. (2007b) Effects of highway forest belts on heavy metals pollution from tail gas of vehicles. *Scientia Silvae Sinicae*, **43**, 1-7.

- WARD N. L., MASTERS G. J. (2007) Linking climate change and species invasion: an illustration using insect herbivores. *Global Change Biology*, **13**, 1605-1615.
- WARREN R. S., BIRCH P. (1987) Heavy metal levels in atmospheric particulates, roadside dust and soil along a major highway. *Science of the Total Environment*, **59**, 253-256.
- WEIGERT K. (2004) National risk assessment: and evaluation of the number and types of Sites of Special Scientific Interest (SSSIs) at risk from road traffic pollution. MSc thesis (Bradford University).
- WELLBURN A. R., HIGGINSON C., ROBINSON D., WALMSLEY C. (1981) Biochemical explanations of more than additive inhibitory effects of low atmospheric levels of sulfur dioxide plus nitrogen dioxide upon plants. *New Phytologist*, **88**, 223-237.
- WHITE R. P., MURRAY S., ROHWEDER M. (2000) *Grassland Ecosystems*. Washington DC, USA: World Resources Institute. Available at: <http://www.wri.org/wr2000>.
- WHITMORE M. E., FREERSMITH P. H. (1982) Growth effects of SO₂ and/or NO₂ on woody plants and grasses during spring and summer. *Nature*, **300**, 55-57.
- WHO (2000) *Air Quality Guidelines for Europe, 2nd edition*. Copenhagen, Denmark: World Health Organisation Regional Publications.
- WILLEMS J. H., PEET R. K., BIK L. (1993) Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. *Journal of Vegetation Science*, **4**, 203-212.

- WILLIAMS N. S. G., MORGAN J. W., MCDONNELL M. J., MCCARTHY M. A. (2005) Plant traits and local extinctions in natural grasslands along an urban–rural gradient. *Journal of Ecology*, **93**, 1203-1213.
- WILSON E. J., WELLS T. C. E., SPARKS T. H. (1995) Are calcareous grasslands in the UK under threat from nitrogen deposition? - an experimental determination of a critical load. *Journal of Ecology*, **83**, 823-832.
- XIA J., WAN S. (2008) Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, **179**, 428-439.
- YACHI S., LOREAU M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 1463-1468.
- YAHDJIAN L., SALA O. E. (2006) Vegetation structure constrains primary production response to water availability in the Patagonian steppe. *Ecology*, **87**, 952-962.
- YIN S., SHEN Z., ZHOU P., ZOU X., CHE S., WANG W. (2011) Quantifying air pollution attenuation within urban parks: An experimental approach in Shanghai, China. *Environmental Pollution*, **159**, 2155-2163.
- ZAVALETA E. S., SHAW M. R., CHIARIELLO N. R., THOMAS B. D., CLELAND E. E., FIELD C. B., MOONEY H. A. (2003) Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecological Monographs*, **73**, 585-604.
- ZECHMEISTER H. G., DULLINGER S., HOHENWALLNER D., RISS A., HANUS-ILLNAR A., SCHARF S. (2006) Pilot study on road traffic

emissions (PAHs, heavy metals) measured by using mosses in a tunnel experiment in Vienna, Austria. *Environmental Science and Pollution Research*, **13**, 398-405.

ZECHMEISTER H. G., HOHENWALLNER D., RISS A., HANUS-ILLAR A. (2005) Estimation of element deposition derived from road traffic sources by using mosses. *Environmental Pollution*, **138**, 238-249.

ZHANG H., SCHRODER J. L., PITTMAN J. J., WANG J. J., PAYTON M. E. (2005) Soil salinity using saturated paste and 1 : 1 soil to water extracts. *Soil Science Society of America Journal*, **69**, 1146-1151.

ZHANG L. X., QIANG H., LI S. Q., CHEN X. L. (2011) Impact of atmospheric ammonia on growth, C and N accumulation and photosynthesis of two maize cultivars with different N root supply. *Plant Soil and Environment*, **57**, 11-18.

Appendix

Appendix 2.1: A summary of regression analyses to show the relationships between net mineralisation rates ($\text{g N m}^{-2} \text{ day}^{-1}$) and net nitrification rates ($\text{g N m}^{-2} \text{ day}^{-1}$) with distance from the roadside in quarters (Q) 1, 2, 3 and 4 and annual mineralisation rates. + denotes that the variable increases with increasing distance from the roadside, – denotes a decline with increasing distance from the roadside and ns denotes non-significance at $p > 0.05$. Logarithmic (log) denotes the pattern of the relationship ($n = 30$).

Site	Variable	Summer	Autumn	Winter	Spring	Annual
High TS	Mineralisation	ns	ns	- log ($t = -3.4$, $p < 0.01$)	ns	ns
	Nitrification	ns	ns	- log ($t = -2.1$, $p < 0.05$)	ns	ns
Medium TS	Mineralisation	- log ($t = -3.4$, $p < 0.01$)	ns	ns	ns	ns
	Nitrification	- log ($t = -3.1$, $p < 0.01$)	ns	ns	ns	ns
Low TS	Mineralisation	ns	ns	ns	ns	ns
	Nitrification	ns	ns	ns	ns	ns

Appendix 2.2: Species present at the high, medium and low trafficked site.

	Low trafficked site	Medium trafficked site	High trafficked site
Grasses			
1	<i>Agrostis capillaris</i>	<i>Agrostis capillaris</i>	<i>Agrostis capillaris</i>
2	<i>Agrostis stolonifera</i>	<i>Agrostis stolonifera</i>	<i>Agrostis stolonifera</i>
3	<i>Arrhenatherum elatius</i>	<i>Arrhenatherum elatius</i>	<i>Arrhenatherum elatius</i>
4	<i>Bromus sterilis</i>	<i>Briza media</i>	<i>Briza media</i>
5	<i>Carex flacca</i>	<i>Bromus hordeaceus</i>	<i>Bromus sterilis</i>
6	<i>Dactylis glomerata</i>	<i>Cynosurus cristatus</i>	<i>Carex flacca</i>
7	<i>Festuca arundinacea</i>	<i>Festuca ovina</i>	<i>Carex spicata</i>
8	<i>Festuca ovina</i>	<i>Festuca rubra</i>	<i>Cynosurus cristatus</i>
9	<i>Holcus lanatus</i>	<i>Helictotrichon pratense</i>	<i>Festuca ovina</i>
10		<i>Holcus lanatus</i>	<i>Holcus lanatus</i>
11		<i>Lolium perenne</i>	<i>Hordeum secalinum</i>
12		<i>Phleum pratense</i>	<i>Koeleria macrantha</i>
13		<i>Poa pratensis</i>	<i>Lolium perenne</i>
14		<i>Vulpia bromoides</i>	
Forbs			
1	<i>Achillea millefolium</i>	<i>Achillea millefolium</i>	<i>Achillea millefolium</i>
2	<i>Agrimonia eupatoria</i>	<i>Bellis perennis</i>	<i>Acinos arvensis</i>
3	<i>Cerastium holosteoides</i>	<i>Cerastium pumilum</i>	<i>Campanula rotundifolia</i>
4	<i>Cirsium arvense</i>	<i>Cirsium arvense</i>	<i>Cirsium acuale</i>
5	<i>Cirsium vulgare</i>	<i>Cirsium vulgare</i>	<i>Cirsium arvense</i>
6	<i>Daucus carota</i>	<i>Clinopodium vulgare</i>	<i>Cirsium vulgare</i>
7	<i>Galium verum</i>	<i>Crepis biennis</i>	<i>Clinopodium vulgare</i>
8	<i>Galium saxatile</i>	<i>Daucus carota</i>	<i>Daucus carota</i>
9	<i>Geranium dissectum</i>	<i>Galium mollugo</i>	<i>Euphrasia nemorosa</i>
10	<i>Geranium molle</i>	<i>Galium saxatile</i>	<i>Galium mollugo</i>
11	<i>Heracleum sphondylium</i>	<i>Galium verum</i>	<i>Galium verum</i>
12	<i>Lathyrus pratensis</i>	<i>Leontodon autumnalis</i>	<i>Gentiana amarella</i>
13	<i>Leontodon hispidus</i>	<i>Leontodon hispidus</i>	<i>Geranium dissectum</i>
14	<i>Linaria vulgaris</i>	<i>Limun catharticum</i>	<i>Geranium molle</i>
15	<i>Lotus corniculatus</i>	<i>Lotus corniculatus</i>	<i>Helianthemum nummularium</i>
16	<i>Medicago lupulina</i>	<i>Medicago lupulina</i>	<i>Hypochaeris glabra</i>
17	<i>Pastinaca sativa</i>	<i>Origanum vulgare</i>	<i>Lathyrus pratensis</i>
18	<i>Pimpinella saxifraga</i>	<i>Pastinaca sativa</i>	<i>Leontodon autumnalis</i>
19	<i>Plantago lanceolata</i>	<i>Pimpinella saxifraga</i>	<i>Leontodon hispidus</i>
20	<i>Prunella vulgaris</i>	<i>Plantago lanceolata</i>	<i>Linum catharticum</i>
21	<i>Ranunculus repens</i>	<i>Potentilla reptans</i>	<i>Lotus corniculatus</i>
22	<i>Sanguisorba minor</i>	<i>Prunella vulgaris</i>	<i>Picris echoides</i>
23	<i>Senecio squalidus</i>	<i>Ranunculus bulbosus</i>	<i>Plantago lanceolata</i>
24	<i>Taraxacum officinale</i>	<i>Sanguisorba minor</i>	<i>Potentilla reptans</i>
25	<i>Thymus polytrichus</i>	<i>Silaceum silaus</i>	<i>Prunella vulgaris</i>
26	<i>Tragopogon pratense</i>	<i>Sucsiza pratensis</i>	<i>Ranunculus bulbosus</i>
27	<i>Trifolium dubium</i>	<i>Taraxacum officinale</i>	<i>Ranunculus repens</i>

28	<i>Trifolium pratense</i>	<i>Thymus polytrichus</i>	<i>Sanguisorba minor</i>
29	<i>Trifolium repens</i>	<i>Trifolium dubium</i>	<i>Tragopogon pratensis</i>
30	<i>Ulex europaeus</i>	<i>Trifolium repens</i>	<i>Trifolium dubium</i>
31	<i>Urtica dioica</i>	<i>Veronica chamaedrys</i>	<i>Trifolium repens</i>
32	<i>Veronica arvensis</i>		<i>Veronica arvensis</i>
33	<i>Veronica chamaedrys</i>		<i>Vicia lathyroides</i>
34	<i>Vicia lathyroides</i>		<i>Viola hirta</i>
35	<i>Vicia spp</i>		
36	<i>Viola hirta</i>		

Shrubs

1	<i>Berberis vulgaris</i>	<i>Crataegus monogyna</i>
---	--------------------------	---------------------------

Moss

1	<i>Amblystegium serpens</i>	<i>Brachythecium rutabulum</i>	<i>Brachythecium rutabulum</i>
2	<i>Homotheicum leutecens</i>	<i>Homotheicum leutecens</i>	
3	<i>Pseudoscleropodium purum</i>	<i>Pseudoscleropodium purum</i>	

Appendix 3.1: Species present (x) at each site.

	AR	BH	LD	MD	NC	SD	SCH	SCP
<u>Grasses</u>								
<i>Agrostis capillaris</i>	x	x	x	x	x	x	x	
<i>Agrostis stolonifera</i>	x	x	x	x	x	x	x	x
<i>Alopecurus pratensis</i>			x					
<i>Arrhenatherum elatius</i>	x	x	x	x	x		x	
<i>Briza media</i>		x					x	
<i>Brachypodium pinnatum</i>					x			
<i>Bromus erectus</i>							x	
<i>Bromus hordeaus</i>		x						
<i>Bromus sterilis</i>	x			x				
<i>Carex carophylla</i>		x			x		x	x
<i>Carex flacca</i>					x			
<i>Carex nigra</i>			x					
<i>Carex spicata</i>	x							
<i>Cynosurus cristatus</i>	x	x						
<i>Dactylis glomerata</i>			x	x	x			
<i>Festuca arundinacea</i>				x				
<i>Festuca ovina</i>	x	x	x	x	x	x	x	x
<i>Festuca rubra</i>		x	x		x			x
<i>Helictotrichon pratense</i>		x				x	x	
<i>Holcus lanatus</i>	x	x	x	x	x	x	x	x
<i>Hordeum secalinum</i>	x							
<i>Koeleria macrantha</i>	x							
<i>Lolium perenne</i>	x	x	x		x	x		
<i>Poa annua</i>								x
<i>Poa trivialis</i>								x
<i>Poa compressa</i>								x
<i>Phleum bertolonii</i>			x					
<i>Phleum pratense</i>		x	x					
<i>Poa pratensis</i>		x						
<i>Trisetum flavescens</i>		x						
<i>Vulpia bromoides</i>		x					x	x
<u>Forbs</u>								
<i>Achillea millefolium</i>	x	x	x	x	x	x	x	
<i>Acinos arvensis</i>	x							
<i>Agrimonia euphatoria</i>			x	x		x	x	
<i>Anacamptis pyramidalis</i>			x					
<i>Anthyllis vulneraria</i>							x	
<i>Anagallis arvensis</i>								x
<i>Aphanes arvensis</i>								x
<i>Arenaria serpyllifolia</i>								x
<i>Bellis perennis</i>		x			x		x	
<i>Campanula glomerata</i>							x	
<i>Campanula rotundifolia</i>							x	
<i>Centaurea nigra</i>					x			
<i>Centaurea scabiosa</i>						x	x	
<i>Cerastium glomeratum</i>						x		x

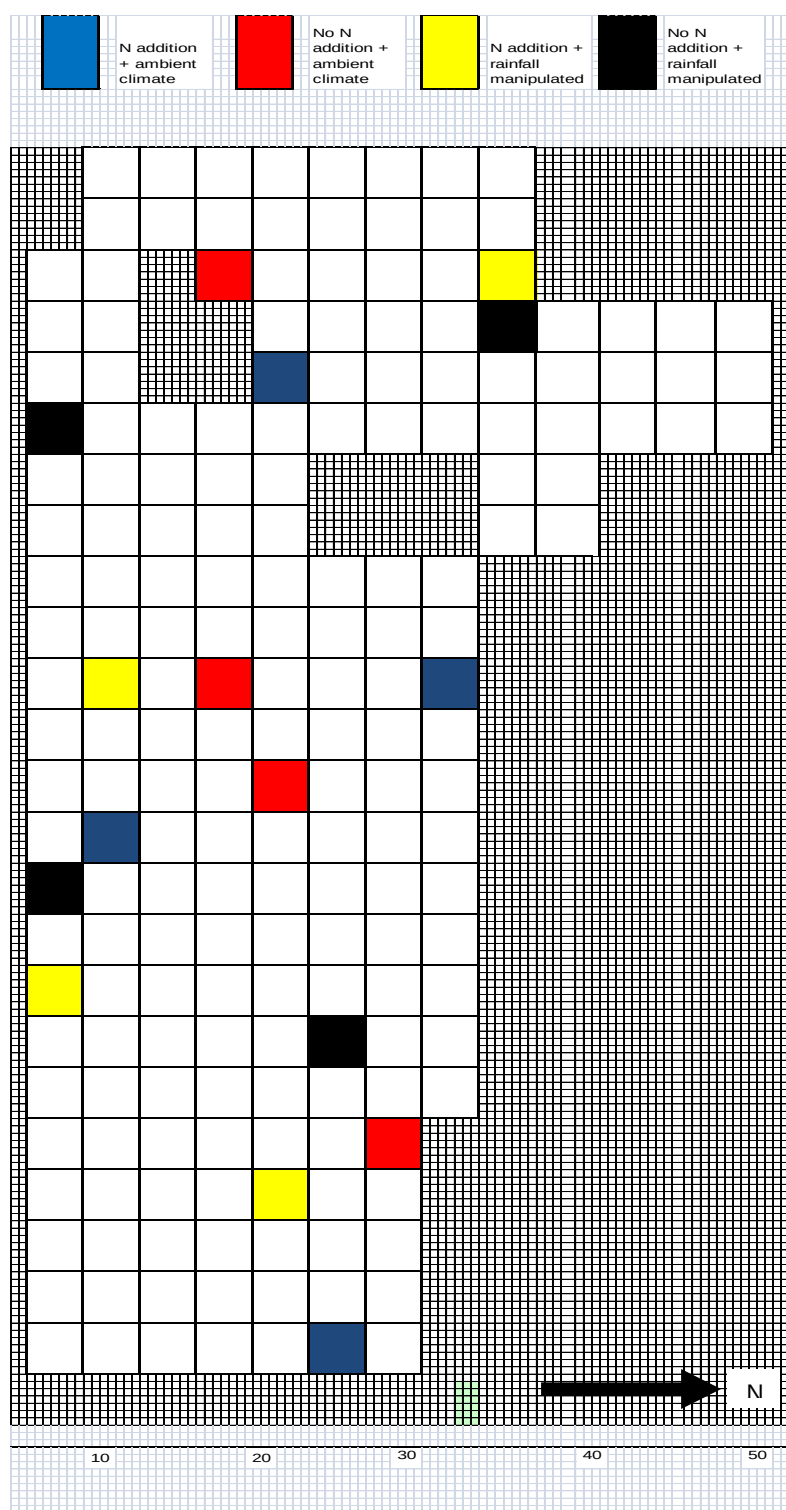
	AR	BH	LD	MD	NC	SD	SCH	SCP
<i>Cerastium holosteoides</i>				X				
<i>Cerastium pumilum</i>		X						
<i>Cirsium acaule</i>	X							X
<i>Cirsium arvense</i>	X	X	X	X	X	X	X	X
<i>Cirsium dissectum</i>			X					X
<i>Cirsium vulgare</i>	X	X	X	X	X			X
<i>Clinopodium vulgare</i>	X	X	X		X	X	X	X
<i>Convolvulus arvensis</i>			X					X
<i>Crepis biennis</i>		X				X		X
<i>Cruciata laevipes</i>					X			
<i>Daucus carota</i>	X	X	X	X	X	X	X	
<i>Epilobium hirsutum</i>					X			X
<i>Euphrasia nemorosa</i>			X		X	X	X	
<i>Filipendula vulgaris</i>							X	
<i>Fragaria vesca</i>								X
<i>Galium mollugo</i>		X	X		X	X	X	X
<i>Galium saxatile</i>		X		X				
<i>Galium verum</i>	X	X	X	X	X	X	X	
<i>Gentiana amarella</i>					X	X	X	
<i>Geranium dissectum</i>	X		X	X				X
<i>Geranium molle</i>	X			X		X	X	X
<i>Glechoma hederacea</i>			X		X	X		X
<i>Helianthemum nummularium</i>							X	
<i>Heracleum sphondylium</i>				X				
<i>Hippocrepis comosa</i>							X	
<i>Hypericum perforatum</i>					X	X	X	X
<i>Hypochaeris radicata</i>								X
<i>Inula conyzae</i>						X		
<i>Lamium galeobdolon</i>						X		
<i>Lathyrus pratensis</i>	X			X	X			
<i>Linaria repens</i>							X	
<i>Linaria vulgaris</i>				X				
<i>Linum catharticum</i>		X	X		X	X	X	X
<i>Leontodon autumnalis</i>		X			X	X		X
<i>Leontodon hispidus</i>		X	X	X	X	X	X	X
<i>Leontodon saxatilis</i>							X	X
<i>Leucanthemum vulgare</i>							X	
<i>Lotus corniculatus</i>	X	X	X	X	X	X	X	X
<i>Medicago lupulina</i>		X		X				X
<i>Medicago minima</i>					X			X
<i>Myosotis arvensis</i>			X					
<i>Myosotis sylvatica</i>								X
<i>Odontites vernus</i>			X			X	X	
<i>Origanum vulgare</i>					X		X	
<i>Pastinaca sativa</i>		X		X				
<i>Picris echioides</i>	X							
<i>Picris hieracioides</i>						X		
<i>Pimpinella saxifraga</i>		X		X				
<i>Plantago lanceolata</i>		X	X	X	X	X	X	
<i>Plantago media</i>						X	X	X
<i>Polygala vulgaris</i>					X			

	AR	BH	LD	MD	NC	SD	SCH	SCP
<i>Potentilla reptans</i>	x	x			x	x		x
<i>Primula veris</i>					x			
<i>Prunella vulgaris</i>		x	x	x	x	x	x	x
<i>Ranunculus bulbosus</i>		x	x		x	x	x	x
<i>Ranunculus repens</i>	x		x	x			x	
<i>Reseda lutea</i>						x		
<i>Rhamnus cathartica</i>								x
<i>Rhinanthus minor</i>					x			
<i>Rumex acetosa</i>			x			x	x	
<i>Rumex acetosella</i>						x		
<i>Sanguisorba minor</i>	x	x		x	x	x	x	
<i>Scabiosa columbaria</i>						x	x	
<i>Senecio jacobaea</i>		x	x	x	x	x		x
<i>Sherardia arvensis</i>								x
<i>Silaum silaus</i>		x						
<i>Silene vulgaris</i>					x			x
<i>Stachys officinalis</i>							x	
<i>Stellaria holostea</i>			x					
<i>Succisa pratensis</i>		x					x	
<i>Taraxacum officinale</i>	x	x	x	x		x	x	x
<i>Thymus polytrichus</i>		x		x				
<i>Tragopogon pratensis</i>	x			x				
<i>Trifolium dubium</i>	x	x	x	x		x	x	x
<i>Trifolium repens</i>	x	x	x	x	x			x
<i>Trifolium pratense</i>		x		x				x
<i>Urtica dioica</i>				x				
<i>Veronica arvensis</i>			x	x	x			x
<i>Veronica chamaedrys</i>	x	x		x	x			x
<i>Veronica serpyllifolia</i>								x
<i>Vicia lathyroides</i>	x			x				
<i>Viola arvensis</i>					x			x
<i>Viola hirta</i>				x	x	x	x	x
<u>Shrub/tree</u>								
<i>Alnus incana</i>					x			
<i>Berberis vulgaris</i>				x				
<i>Crataegus monogyna</i>	x				x	x	x	x
<i>Euonymus europaeus</i>					x			
<i>Fraxinus excelsior</i>								x
<i>Prunus spinosa</i>								x
<i>Rubus fruticosus</i>			x		x	x		x
Grass species richness	11	16	12	8	11	6	10	9
Forb species richness	22	31	30	34	37	37	40	44
Shrub species richness	1	0	1	1	4	2	1	4
Total species richness	34	47	43	43	52	45	51	57

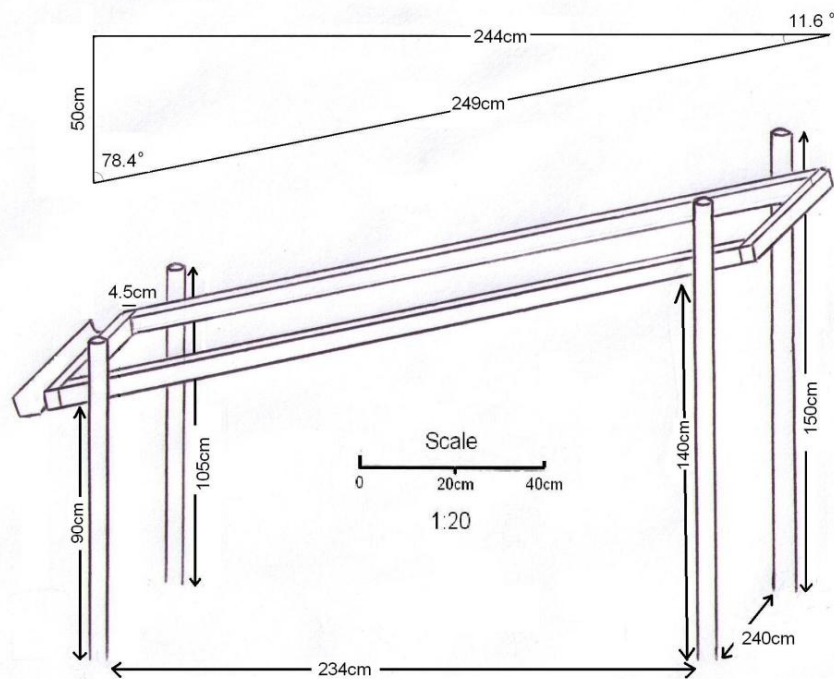
Appendix 3.2: Summary of optimal mixed effects model output across all study sites. Terms in initial models: log distance (D), traffic volume (vol) and their interaction. Ns denotes non-significance at $p > 0.05$.

HM	Optimal Model Terms	n	t	p	HM	Optimal Model Terms	n	t	p
As	na			ns	Ni	Intercept	69	2.5	< 0.05
Cd	na			ns		log (D)		2.3	< 0.05
Co	na			ns		Vol		1.5	ns
Cr	Intercept	68	1.6	ns		Vol x log (D)		-2.0	< 0.05
	log (D)		3.5	< 0.001	Pb	Intercept	69	12.5	< 0.001
	Vol		1.4	ns		log (D)		-4.7	< 0.001
	Vol x log (D)		-3.0	< 0.01	V	Intercept	69	2.3	< 0.05
Cu	Intercept	69	6.7	< 0.01		log (D)		3.1	< 0.01
	log (D)		-1.6	ns		Vol		1.4	ns
	Vol		2.8	< 0.05		Vol x log (D)		-2.8	< 0.01
	Vol x log (D)		-3.0	< 0.01	Zn	Intercept	69	8.7	< 0.001
						log (D)		-3.7	< 0.001

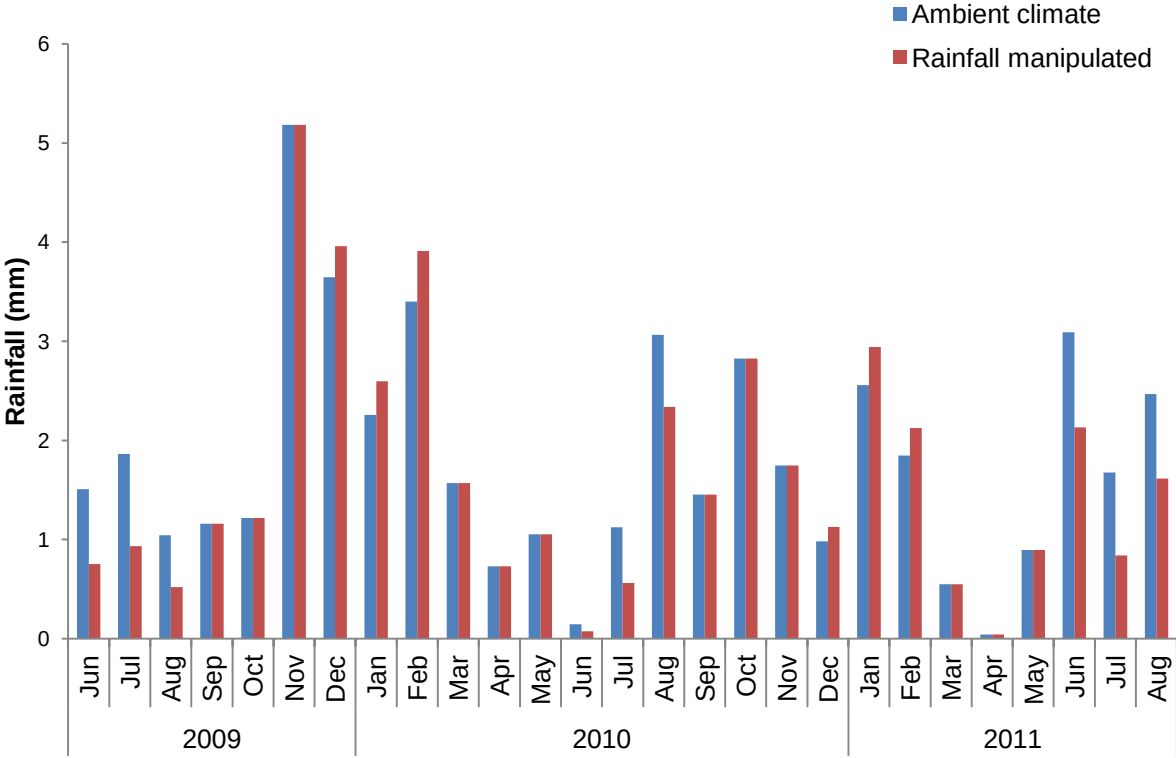
Appendix 4.1: Schematic representation of the study site with colours indicating treatments.



Appendix 4.2: Diagram of shelters used during summer (JJA) which were directed toward the prevailing wind. Climate change plots had transparent corrugated plastic roofs on top of these structures to direct precipitation to the guttering where it was collected. Current climate plots had transparent corrugated plastic roofs on top with holes in to allow precipitation to percolate through (adapted from Fry, 2011).



Appendix 4.3: Monthly rainfall throughout the experimental period for rainfall manipulated and ambient climate plots. Weather data was obtained using an onsite Vantage Pro wireless weather station (Davis Instruments, USA).



Appendix 4.4: Power Analysis of above-ground biomass (AGB) and below-ground biomass (BGB) in year one. Between group and within group variance is also presented. Factors are nitrogen enrichment (N), rainfall manipulated (rainfall) and the interaction between rainfall and nitrogen enrichment (rainfall:N)

Factor	Between Group	Within Group	Power
<u>AGB</u>			
N	0.5	69.1	0.05
Rainfall	20.9	58.6	0.29
Rainfall:N	14.5	60.2	0.20
<u>BGB</u>			
N	3694.0	37754.0	0.11
Rainfall	217.8	39565.0	0.05
Rainfall:N	3063.2	39104.0	0.09