

Plant and arthropod community sensitivity to rainfall manipulation but not nitrogen enrichment in a successional grassland ecosystem

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Abstract Grasslands provide many ecosystem services including carbon storage, biodiversity preservation and livestock forage production. These ecosystem services will change in the future in response to multiple global environmental changes, including climate change and increased nitrogen inputs. We conducted an experimental study over 3 years in a mesotrophic grassland ecosystem in southern England. We aimed to expose plots to rainfall manipulation that simulated IPCC 4th Assessment projections for 2100 (+15 % winter rainfall and –30 % summer rainfall) or ambient climate, achieving +15 % winter rainfall and –39 % summer rainfall in rainfall-manipulated plots.

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Nitrogen ($40 \text{ kg ha}^{-1} \text{ year}^{-1}$) was also added to half of the experimental plots in factorial combination. Plant species composition and above ground biomass were not affected by rainfall in the first 2 years and the plant community did not respond to nitrogen enrichment throughout the experiment. In the third year, above-ground plant biomass declined in rainfall-manipulated plots, driven by a decline in the abundances of grass species characteristic of moist soils. Declining plant biomass was also associated with changes to arthropod communities, with lower abundances of plant-feeding Auchenorrhyncha and carnivorous Araneae indicating multi-trophic responses to rainfall manipulation. Plant and arthropod community composition and plant biomass responses to rainfall manipulation were not modified by nitrogen enrichment, which was not expected, but may have resulted from prior nitrogen saturation and/or phosphorus limitation. Overall, our study demonstrates that climate change may in future influence plant productivity and induce multi-trophic responses in grasslands.

Keywords Biodiversity · Climate change · Drought · Ecosystem functioning · Nitrogen addition

Introduction

The global climate, as well as the nitrogen (N) and carbon (C) cycles, is projected to be strongly altered by anthropogenic activities in the future (IPCC 2007, 2008, 2013; Galloway et al. 2008). Many natural ecosystems, including grasslands, will therefore experience novel environmental conditions, with unknown consequences for their capacity to provide important ecosystem services including C-sequestration, biodiversity preservation and the provision of livestock forage (Sala et al. 2000; Millennium

Ecosystem Assessment 2006). Estimates of the capacity for grasslands to continue to provide these benefits are associated with uncertainty, in part because there are few quantitative data describing how ecosystems will respond to multiple and potentially interacting global change drivers (Beier et al. 2004; Trumper et al. 2009; Lee et al. 2010).

Climate models predict that many of the world's ecosystems (particularly northern temperate zones) will experience changing rainfall patterns and amounts, including an increased frequency of drought events (IPCC 2008). Grasslands may show particularly strong responses to these changes because there is evidence that their annual net primary productivity responds more strongly to variability in rainfall than other biomes (Knapp and Smith 2001). Studies have also shown that both experimental and natural drought can reduce grassland plant biomass production, change plant species composition and cause localised extinctions (Tilman and Haddi 1992; Laporte et al. 2002; Morecroft et al. 2004). However, there is substantial variation between sites, with productivity declines in some grasslands occurring within 1 year of rainfall reduction (Tilman and Haddi 1992), while the productivity of others persists despite reduced rainfall for over a decade (Grime et al. 2000). Impacts of drought on the plant community may also affect the wider community and the ecosystem services they provide. Such changes have received little attention but may be highly significant. For example, declines in the abundance of phloem-feeding insects have been associated with rainfall reductions as their feeding becomes inhibited by reduced plant cell turgor (Mattson and Haack 1987; Huberty and Denno 2004). Furthermore, declines in the abundance of plant-feeding arthropods have been associated with reduced plant tissue N concentrations resulting from lower rainfall (Lauenroth et al. 1978).

Any changes to ecosystems caused by climate change will occur alongside the effects of other global change drivers, of which nitrogen enrichment is one of the most significant (Sala et al. 2000; Millennium Ecosystem Assessment 2006; Manning 2012). Direct fertiliser applications can be substantial: in 2000, 11 % of the world's natural vegetation received N deposition greater than $10 \text{ kg ha}^{-1} \text{ year}^{-1}$ (Dentener et al. 2006). Globally, both fertiliser application and reactive N emissions are continuing to rise, with substantial areas of the world projected to receive N deposition inputs in excess of $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ by 2050 (Galloway et al. 2004). Plant biomass gains in response to these future increases in N inputs are anticipated, since the productivity of almost all biomes is N-limited, and such effects have been widely reported (LeBauer and Treseder 2008). A shift in resource allocation by plants in response to N enrichment may also be expected in grasslands, through increased above-ground growth and reduced or unchanged root growth (Lee et al. 2010). Declines in regional and

local plant biodiversity and changes to community composition are also associated with increased N inputs (Stevens et al. 2004, 2006, 2011; Bobbink et al. 2010; Southon et al. 2013). Less well understood are the multi-trophic impacts of these changes, which are potentially large (Manning, 2012). Increases in the abundance of herbivorous sap-feeding arthropods and carnivorous arthropods (spiders) have been associated with N enrichment, with most of the variation driven by increased plant productivity (Boyer et al. 2003; Cole et al. 2008), thus indicating a bottom-up trophic cascade. In contrast, another study demonstrated suppression of insect herbivore populations by the increase in predation that occurs when habitats become more suitable for predatory invertebrates (tall and dense plant canopy) as a result of N enrichment (Fraser and Grime 1998).

The interaction between drought and N enrichment and the effect of this interaction on ecosystems is potentially large, but has seldom been measured experimentally. Soil moisture content strongly controls N availability to plants since N uptake by roots is primarily from solution (Borken and Matzner 2009). This effect could see N enrichment effects suppressed by drought. N deposition has frequently been associated with reduced root:shoot ratios (Lee et al. 2010), so there is considerable potential for interactions between these two global change drivers. Indeed, meta-analysis has shown that plant biomass responses to N enrichment depend on local climate, with lower biomass responses to N enrichment in low rainfall regions (Lee et al. 2010). The few experiments that have investigated the response of grasslands to simultaneous manipulations of N supply and rainfall regime indicate co-limitation. For example, above-ground productivity increases in a Californian 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, we are not aware of any published studies in grasslands that have investigated the simultaneous effects of both reduced rainfall and N enrichment (Bobbink et al. 2010). This has only been investigated in heathland, where a combination of N addition and rainfall reduction increased the likelihood of weed invasion by increasing the productivity of grasses (Bobbink et al. 1998). This paucity of information extends to arthropod communities.

Our study sought to address the knowledge gap concerning N and drought interactions in grasslands by simulating projected future (2100) rainfall and N enrichment patterns. These were manipulated in factorial combination in a mesotrophic grassland in the UK and a range of ecosystem properties (some community level, some biogeochemical) were measured. The following hypotheses were tested: (1) soil moisture content, plant productivity, herbivorous

insects and their predators will decline in response to summer drought, with an associated change in plant community composition; (2) soil N availability and plant productivity would increase under N enrichment, with associated increases in nitrophilic plant species, sap-sucking arthropod species and their predators; and (3) plant biomass increases and plant and arthropod community shifts in response to nitrogen manipulation will not occur when N addition is combined with rainfall manipulation, because reduced soil moisture during the growing season will limit growth responses and reduce plant uptake of N.

Materials and methods

Site and treatments

The study took place from May 2009 to September 2011 in a grassland classified as a MG6: *Lolium perenne*/*Cynosaurus cristatus* grassland in the UK National Vegetation Classification (Rodwell 1992) at Silwood Park, Ascot, UK (51.507N, 0.640W). Mean annual temperature and rainfall over the four calendar years prior to the experiment was 11 °C and 741 mm year⁻¹, respectively. During the 4 years prior to the experiment, mean summer (June–August) rainfall was 140.6 mm and mean winter (November–January) rainfall was 167.6 mm. In 2009 (135.3 mm) and 2010 (134.2 mm), summer rainfall approximated the 4-year mean, whereas in 2011, summer rainfall was considerably higher (221.2 mm). In the first winter (2009/10), rainfall was approximately 66 % higher than the 4-year seasonal average, while in the second winter (2010/11), rainfall was approximately equal to it (161.4 mm; for more information on meteorological conditions, see Supplementary Appendix 1). Site weather data were obtained using an onsite Vantage Pro weather station (Davis Instruments, USA).

The site was ploughed in October 2007, which removed the majority of the standing biomass. It was then left undisturbed to regenerate naturally. Dominant plant species over the duration of the study were the grasses *Agrostis capillaris*, *Arrhenatherum elatius* and *Holcus mollis*, the forb *Cirsium arvense*, and the legume *Trifolium repens*. The site is situated upon tertiary sandy deposits (the Bagshot Beds) with overlying soils, typically a layer of sandy deposits to a depth of approximately 1 m covering a layer of bleached silica and hydrous ferric oxides (Crawley 2005). The dominant soil type was sandy loam with a pH of approximately 5.5.

A fully factorial, randomised block design was used with 16, 2.4 m × 2.4 m, plots that were located within the design of a larger experiment investigating the influence of plant diversity on ecosystem responses to changes in rainfall (Fry

et al. 2013, 2014a) (see Supplementary Appendix 2 for site layout). The four blocks traversed a slight incline at the site that correlated with variation in soil moisture and species composition (Fry et al. 2013). Treatments were N enrichment (0 or 40 kg ha⁻¹ year⁻¹, here N⁰ and N⁺) and rainfall manipulation (ambient climate or 2100 predictions, the latter referred to here as rainfall-manipulated—outlined below) arranged in a factorial design with four treatments: (1) N enrichment only (ambient climate, N⁺), (2) N 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 the four blocks, giving a total of 16 plots.

Rainfall manipulations simulated regional end-of-century (2100) rainfall projections for the region (IPCC 2007; Murphy et al. 2009) Summer rainfall is predicted to decline by ~30 % in southeast England, with an increased frequency of drought periods and downpour events. Winter 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. Ambient climate plots were covered with transparent corrugated plastic roofs with holes in that allowed rain to pass through to the plots below (for a diagram of the roof design, see Supplementary Appendix 3). In rainfall-manipulated plots, all collected rainfall was applied if more than 20 mm fell over a 24-h period, and 50 % of rainfall was applied if less than 20 mm fell. This was designed to emulate the projected cycle of drought interspersed with heavy downpour events, while also reducing total rainfall by 30 % (Murphy et al. 2009). Over the three summers an average of 61 % of summer rainfall was reapplied (2009: 50 %; 2010: 69 %; 2011: 63 %), 9 % less than the target amount. In winter, plots receiving the rainfall manipulation treatment received an additional 15 % of rainfall. This was collected in trays of 15 % of the plot area that were placed adjacent to the plots. In both 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 course of the experiment the combination of summer and winter treatments resulted in rainfall-manipulated plots receiving 91 % of the annual rainfall received by ambient climate plots.

Annual background total (wet + dry) N deposition rates at the study site are estimated to be 18 kg N ha⁻¹ year⁻¹, of which approximately 50 % represents oxidised N deposition and 50 % reduced N deposition (RoTAP 2011). We are not aware of any further N inputs prior to the start of the experiment. The critical load for this site is estimated to be 15–20 kg N ha⁻¹ year⁻¹ and when this load is exceeded graminoids are expected to increase in abundance, alongside declines in the abundance of forbs and species richness

(Bobbink et al. 2010). Nitrogen, in the form of ammonium nitrate, was applied twice monthly at a rate of $40 \text{ kg ha}^{-1} \text{ year}^{-1}$ to the N^+ treatment plots, hence total N deposition in the N-enriched plots was estimated to be $58 \text{ kg ha}^{-1} \text{ year}^{-1}$. This is within the range of N deposition rates received by European non-forest ecosystems in 2000 (Den-tener et al. 2006), but lower than what is often applied as fertiliser to agricultural grasslands (Blüthgen et al. 2012). For applications, ammonium nitrate was dissolved in 5 L of rainfall, which had been previously collected at the site (concentration = 7 mmol). At the same time, 5 L of rainfall with no additional N was added to the N^0 plots to ensure consistency of the rainfall received by the N^+ and N^0 plots, adding approximately 0.1 % of the ambient rainfall received by each plot per fortnightly application.

Soil moisture, extractable nitrogen and mineralisation

PVC cores (2.5 cm diameter, 15 cm length) were used to collect soil samples from each plot at six time intervals: June, September and December 2009, March and June 2010, and June 2011, with sampling designed to monitor seasonal fluctuations. PVC cores were immediately reintroduced into the ground adjacent to the original sampling location, covered with adhesive tape to prevent leaching losses, and to prevent contamination from experimental N additions, then left for 3 months before the soil was collected (incubated sample). Inorganic oxidised N ($\text{NO}_2^- + \text{NO}_3^-$) and reduced N (NH_4^+) were extracted from a 10-g sub-sample of both incubated (T_1) and fresh (T_0) soil by mixing with 37.5 mL of 1 M KCl; concentrations were measured using a SKALAR San Flow⁺⁺ auto-analyser (SKALAR, Netherlands). A further 5-g sub-sample was dried in an oven for 48 h at $80 \text{ }^\circ\text{C}$ ($\pm 10 \text{ }^\circ\text{C}$), with the relative mass loss taken as the soil moisture content. SKALAR outputs were adjusted for soil moisture content to give extractable N concentrations per unit dry soil (mg kg^{-1}). Ten randomly positioned spot measurements of soil moisture in the upper 5 cm of the soil profile were taken weekly in summer 2011 using a ThetaProbe soil moisture sensor (Delta-T, UK).

Net mineralisation rates (MR) and net nitrification rates (NR) for summer (June–September 2009), autumn (September–December 2009), winter (December 2009–March 2010) and spring (March–June 2010) were estimated from the extractable N content of incubated (t_1) and fresh soil samples (t_0) 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 ($\text{g m}^{-2} \text{ year}^{-1}$). 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. $\text{MR} = \text{total extractable N } (t_1) - \text{total extractable N } (t_0) \times \text{bulk density.}$
2. $\text{NR} = \text{extractable oxidised N } (t_1) - \text{extractable oxidised N } (t_0) \times \text{bulk density.}$

Plant composition and biomass

Cover estimates of vascular plants were made using $1 \text{ m} \times 1 \text{ m}$ quadrats placed in the central 1 m^2 of each plot. All vascular plants were identified to species level and their percentage cover was estimated. Vegetation surveys took place in July 2009, July 2010 and July 2011.

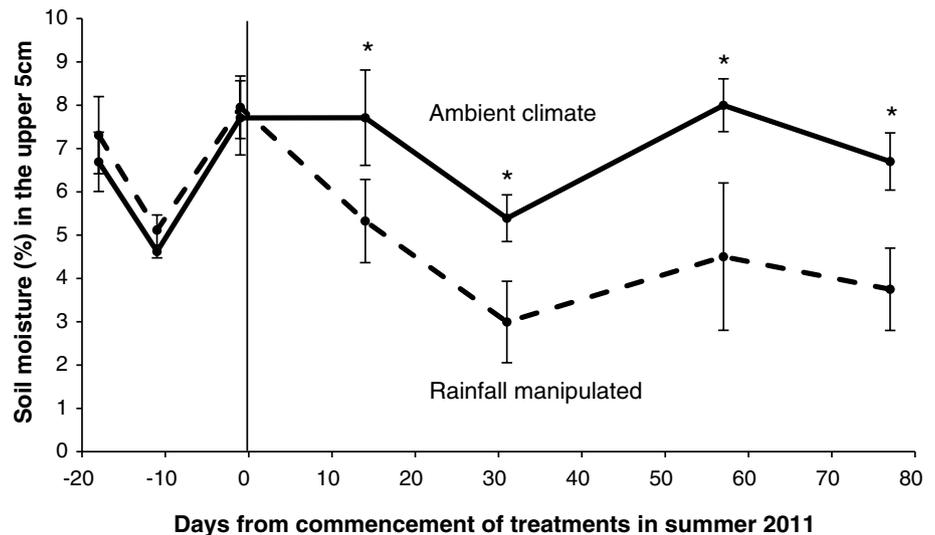
Destructive harvests were carried out in September 2011 when many species were at maximum biomass. Vegetation from four $25 \text{ cm} \times 25 \text{ cm}$ randomly positioned sub-samples (representing 25 % of the central 1 m^2 plot area) was cut to ground level and removed from all 16 plots. Samples were dried for 48 h, litter was removed and functional groups were separated 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 and averaging these.

Root growth was measured by installation of 4 nylon mesh cores ($780 \text{ } \mu\text{m}$ diameter mesh, 15 cm length, 2.5 cm width) per plot in May 2010 and May 2011. Each core contained sieved, root-free soil which had been collected from that plot on the same day as they were introduced. Root cores were removed after 3-month periods in both years (July 2010 and 2011). Roots that had grown into the cores were extracted, washed, dried and weighed.

Arthropod community

Arthropod sampling took place during two 1-week periods in July 2010 and July 2011. All samples were collected between 1200 and 1500 hours on dry days to ensure that sampling conditions were as consistent as possible. 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 m^2 of each plot. The suction duration was 16 s, which has been demonstrated to collect 90 % of the aboveground invertebrates present in grassland (Brook et al. 2008). Four replicates within each plot meant that 16 % of the central 1 m^2 of each plot was sampled. From the samples, Auchenorrhyncha, Araneae, Coleoptera, Collembola, Diptera, Heteroptera, Hymenoptera and Isopoda were identified, separated and counted. Chilopoda, Lepidoptera, Myriapoda, Opiliones, Orthoptera and Thysanoptera were also recorded and counted, but were of insufficient abundance to warrant statistical analysis. Acari and Sternorrhyncha were also recorded at the site, but their numbers were too high to be reliably counted and have therefore been omitted.

Fig. 1 Rainfall treatment effects on soil moisture (%) during summer 2011. Values are the means of 3 measurements taken from each of the 8 rainfall-manipulated (*dashed line*) and 8 ambient climate plots (*continuous line*) at each time point (\pm SE). The vertical line represents the start of summer rainfall treatments. Asterisk $P < 0.05$



Statistical analyses

The effect of the treatments on extractable N, mineralisation rates, nitrification rates, plant cover estimates, insect abundance counts, above- and below-ground biomass and plant and insect species diversity were assessed using two-way ANOVA in R (v.3.0.1, <http://www.r-project.org>). Data were tested for normality using a Shapiro test. Where appropriate response variables were log- or square root-transformed to achieve normality prior to statistical testing. Climate and N treatments and the interaction between both variables were included in the initial statistical models as explanatory variables, along with a block term (Crawley 2007). This block term was subsequently removed from all the statistical models since it was never significant ($P > 0.05$ in all cases).

Results

Soil moisture, nitrogen availability and turnover rates

There were no differences between any of the treatments in the soil moisture content of the upper 15 cm of the soil profile for any of the quarterly spot measurements over the 3 years of the experiment (all $P > 0.05$). In the final summer (2011), soil moisture content in the upper 5 cm of the soil profile was also measured and was found to decline in rainfall-manipulated plots within 2 weeks of commencement of the summer treatments (Fig. 1). This difference between rainfall-manipulated and ambient climate plots persisted throughout the treatment period (June–August 2011). N addition did not affect soil moisture content at any time in the 3 years experiment ($P > 0.05$).

Annual mineralisation rates in the year ending June 2010 were increased by 50 % in N^+ plots compared with N^0 plots (Fig. 2a). Despite a large effect size ($F = 3.7$), these differences were not significant ($P = 0.088$; Table 1). Quarterly mineralisation rates from July 2009 to June 2010 did not differ between treatments ($F = 0.003$ –2.7, all $P > 0.05$; Supplementary Appendix 4). However, elevated annual rates of mineralisation in N^+ plots was associated with a significant increase in annual rates of nitrification which contributed 70 % of annual net rates of mineralisation across the plots. Net annual rates of nitrification in N^+ plots were 90 % higher than in the controls ($F = 10.5$, $P = 0.010$; Fig. 2b), an effect that was primarily driven by summer increases; June 2009–August 2009 ($F = 5.7$, $P = 0.041$). Rainfall manipulation, both on its own and in combination with N addition, never demonstrably influenced mineralisation or nitrification rates in any other period ($F = 0.001$ –2.7, all $P > 0.05$; for a complete list of results, see Supplementary Appendix 4).

Individual spot measurements of extractable oxidised N revealed that concentrations were strongly elevated in N^+ ($8.2 \pm 1.9 \text{ mg kg}^{-1}$) compared with N^0 plots ($3.5 \pm 1.3 \text{ mg kg}^{-1}$) in autumn 2009, but, despite a large effect size ($F = 4.0$), this change was not statistically significant ($P = 0.068$). At all other times, treatment effects on extractable oxidised N were not significant, and there were also no significant treatment effects on extractable reduced N at any time during the study ($F = 0.03$ –2.5, all $P > 0.05$; Supplementary Appendix 4).

Species richness, plant composition and biomass production

The vast majority of total plant biomass was made up of grasses (mean 96 %) at the site. Across all plots, mean

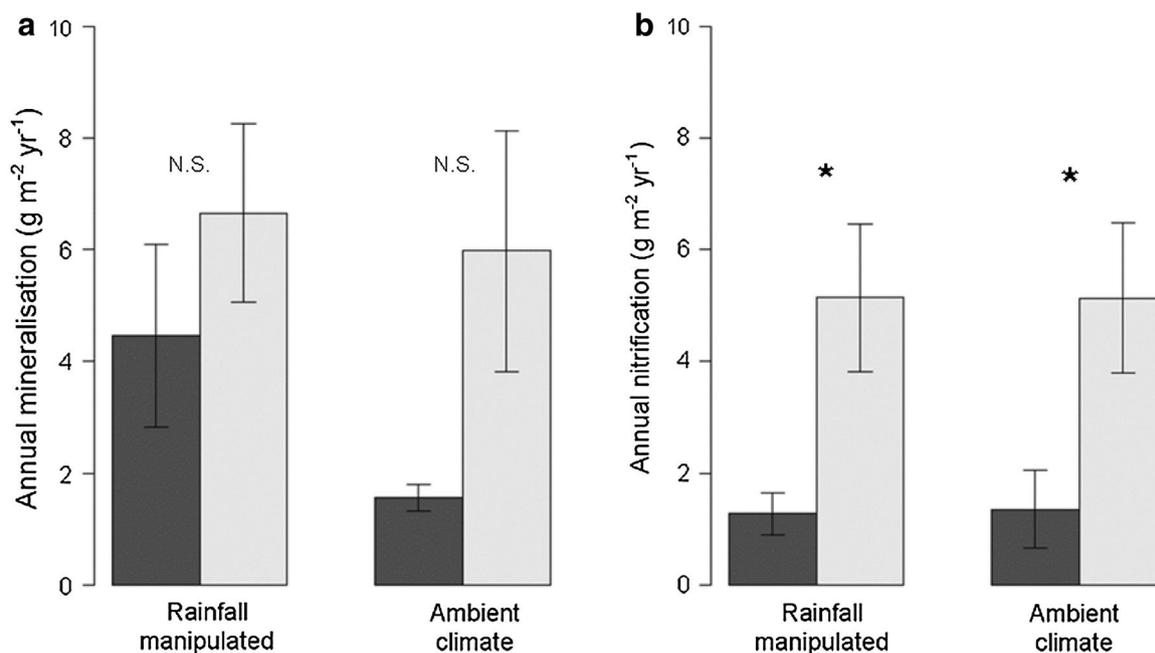


Fig. 2 Annual rates of **a** mineralisation and **b** nitrification rates in nitrogen addition (*dark bars*) and ambient nitrogen (*light bars*) treatments. Values are the means of four replicates (\pm SE). *Asterisk*

$P < 0.05$ and *NS* not significant for nitrogen addition effects. There were no significant effects of rainfall manipulation ($P > 0.05$)

vegetation height—used as a proxy for aboveground biomass—was 10.7 and 13.2 cm in 2009 and 2010, respectively. Vegetation height was 0.3 % (2009) and 14 % (2010) lower in rainfall-manipulated plots in these years. Although treatment effects on vegetation height were not significant in 2010 ($F = 0.1$ – 0.5 , $P > 0.05$), there was an overall trend towards significance over the treatment years with a P value of 0.1 by the third year ($F = 2.7$). In 2011, total above-ground biomass was also assessed by harvesting. This had declined by 28 % in rainfall-manipulated plots ($16.8 \pm 3.4 \text{ g m}^{-2}$) compared with ambient climate plots ($23.3 \pm 4.2 \text{ g m}^{-2}$, $F = 11.1$, $P = 0.001$); this difference was driven by a 30 % decline in grass biomass in rainfall-manipulated plots ($15.8 \pm 3.6 \text{ g m}^{-2}$) compared with ambient climate plots ($22.7 \pm 4.3 \text{ g m}^{-2}$, $F = 11.8$, $P = 0.001$; Table 1). Forb (mean across all treatments = $1.5 \pm 0.7 \text{ g m}^{-2}$) biomass was not affected by any of the treatments ($F = 1.1$ – 1.5 , $P > 0.05$).

Mean root growth over the 3-month periods across all plots was 1.6 and 3.5 g cm^{-2} in 2009 and 2010, 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 (2010). This effect was pronounced ($F = 3.8$) but not significant ($P = 0.059$). In the third treatment year, there was no difference in root biomass between rainfall-manipulated plots and ambient plots ($F = 0.1$, $P = 0.725$). N enrichment did not result in any changes to above- or below-ground biomass accumulation throughout the experiment

($F = 0.1$ – 1.4 , all $P > 0.05$). There were also no effects of the combined rainfall manipulation and N addition treatment on above- or below-ground biomass accumulation ($F = 0.2$ – 1.5 , all $P > 0.05$). Treatment effects on the cover of grasses or forbs, or the ratio of grasses to forb cover in 2009 and 2010 were all non-significant ($F = 0.02$ – 1.1 , all $P > 0.05$). In 2011, there was a 9 % decline in grass cover and a marked 70 % increase in the forb cover of rainfall-manipulated plots when compared with ambient climate plots. Whilst the changes in grass and forb cover were not statistically significant, the effect sizes were considerable, reaching $F = 2.7$ ($P = 0.123$) and $F = 2.5$ ($P = 0.143$) in 2011, respectively. At this time, destructive harvests demonstrated that grass biomass had declined significantly in rainfall-manipulated plots ($F = 11.8$, $P = 0.001$). This decline was driven by a decrease in the total combined cover of the dominant three species: *Agrostis capillaris*, *Arrhenatherum elatius* and *Holcus mollis* ($F = 4.9$, $P = 0.010$; Fig. 3). In previous years, the total combined cover of these species was not significantly affected ($F = 0.003$ – 1.2 , $P > 0.05$). The coverage of individual forb and grass species were not related to any of the treatments ($F = 0.01$ – 1.9 , all $P > 0.05$).

Arthropod community

There was approximately double the number of Hymenoptera in rainfall-manipulated plots in the second year of the

Table 1 ANOVA analyses summarising plant responses to nitrogen (*N*) or rainfall manipulation (*Rainfall*) when compared with controls (no *N* and ambient climate)

Response	2009			2010			2011			
	Treatment	<i>F</i>	<i>P</i>	Treatment	<i>F</i>	<i>P</i>	Treatment	<i>F</i>	<i>P</i>	
Grass biomass	N	–	–	N	–	–	N	0.100	0.753	
	Rainfall	–	–	Rainfall	–	–	Rainfall	11.753	0.001	–
	N:Rainfall	–	–	N:Rainfall	–	–	N:Rainfall	0.021	0.885	
Forb biomass	N	–	–	N	–	–	N	1.299	0.259	
	Rainfall	–	–	Rainfall	–	–	Rainfall	1.147	0.288	
	N:Rainfall	–	–	N:Rainfall	–	–	N:Rainfall	1.488	0.227	
Total biomass	N	–	–	N	–	–	N	0.284	0.596	
	Rainfall	–	–	Rainfall	–	–	Rainfall	11.102	0.001	–
	N:Rainfall	–	–	N:Rainfall	–	–	N:Rainfall	0.138	0.712	
Canopy height	N	–	–	N	0.496	0.495	N	1.395	0.239	
	Rainfall	–	–	Rainfall	0.254	0.623	Rainfall	2.718	0.100	–
	N:Rainfall	–	–	N:Rainfall	0.074	0.791	N:Rainfall	0.405	0.525	
Root biomass	N	–	–	N	0.267	0.608	N	2.093	0.156	
	Rainfall	–	–	Rainfall	3.763	0.059	Rainfall	0.125	0.725	+
	N:Rainfall	–	–	N:Rainfall	0.102	0.751	N:Rainfall	0.414	0.524	
Grass cover	N	0.138	0.717	N	0.037	0.850	N	0.341	0.570	
	Rainfall	0.228	0.642	Rainfall	1.060	0.324	Rainfall	2.749	0.123	
	N:Rainfall	0.161	0.302	N:Rainfall	0.017	0.900	N:Rainfall	0.104	0.314	
Forb cover	N	0.361	0.559	N	0.022	0.885	N	0.613	0.449	
	Rainfall	1.003	0.336	Rainfall	0.300	0.419	Rainfall	2.454	0.143	
	N:Rainfall	0.617	0.447	N:Rainfall	0.002	0.962	N:Rainfall	1.452	0.251	
Species richness	N	0.017	0.899	N	0.028	0.870	N	1.263	0.283	
	Rainfall	0.424	0.527	Rainfall	1.374	0.264	Rainfall	0.316	0.584	
	N:Rainfall	1.373	0.264	N:Rainfall	0.252	0.625	N:Rainfall	1.263	0.283	
Annual mineralisation	N	3.651	0.088	+	N	–	–	N	–	–
	Rainfall	0.951	0.355		Rainfall	–	–	Rainfall	–	–
	N:Rainfall	0.397	0.544		N:Rainfall	–	–	N:Rainfall	–	–
Annual nitrification	N	10.530	0.010	+	N	–	–	N	–	–
	Rainfall	0.001	0.981		Rainfall	–	–	Rainfall	–	–
	N:Rainfall	0.001	0.971		N:Rainfall	–	–	N:Rainfall	–	–

Bold denotes significance at $P < 0.05$. Direction of responses presented as increasing (+) or decreasing (–) compared to control for a P of greater than or equal to 0.1 for indicative purposes; – indicates not measured

experiment, with a mean of $89 \pm 5 \text{ m}^{-2}$ individuals in rainfall-manipulated plots compared to $46 \text{ individuals} \pm 15 \text{ m}^{-2}$ in ambient climate plots. There were no treatment effects on any of the other arthropod groups; Auchenorrhyncha (mean $325 \text{ individuals m}^{-2}$), Araneae (mean $63 \text{ individuals m}^{-2}$), Coleoptera (mean $34 \text{ individuals m}^{-2}$), Collembola ($4,582 \text{ individuals m}^{-2}$), Diptera ($29 \text{ individuals m}^{-2}$), Heteroptera (mean $26 \text{ individuals m}^{-2}$) and Isopoda (mean $7 \text{ individuals m}^{-2}$) in the second year of the experiment ($F = 0.001\text{--}2.3$, all $P = 0.2\text{--}1.0$ in 2010; Supplementary Appendix 5). In the third year, there was a 40 % decline in the number of Auchenorrhyncha ($F = 8.2$, $P = 0.014$), with an average of $406 \text{ individuals} \pm 46 \text{ m}^{-2}$ in the ambient climate plots

and $244 \text{ individuals} \pm 36 \text{ m}^{-2}$ in the rainfall-manipulated plots (Fig. 4a). Heteroptera abundance was not altered by rainfall manipulation or N inputs at any stage ($F = 0.003\text{--}1.9$, $P = 0.20\text{--}0.96$). There was also a 33 % decline in the number of Araneae in rainfall-manipulated plots ($F = 5.6$, $P = 0.035$), with an average of $75 \text{ individuals} \pm 7 \text{ m}^{-2}$ in the ambient climate plots and $50 \text{ individuals} \pm 8 \text{ m}^{-2}$ in the rainfall-manipulated plots (Fig. 4b). Hymenoptera increased by 12 % in N^+ plots ($F = 4.8$, $P = 0.048$), with a mean of $169 \text{ individuals} \pm 19 \text{ m}^{-2}$ in N^+ and $148 \text{ individuals} \pm 36 \text{ m}^{-2}$ in N^0 plots (Fig. 4c). The abundances of individuals from other insect groups were not related to any of the treatments in the third year ($F = 0.01\text{--}2.1$, all $P = 0.17\text{--}0.97$).

Fig. 3 Mean cover (%) of the three dominant grass species; *Agrostis capillaris* (filled black), *Arrhenatherum elatius* (dots) and *Holcus mollis* (diagonal lines) in rainfall-manipulated or ambient climate treatments, with ambient nitrogen or nitrogen addition plots in factorial combination. Values are means of four replicates (\pm SE). Asterisk $P < 0.05$ and NS not significant ($P > 0.05$)

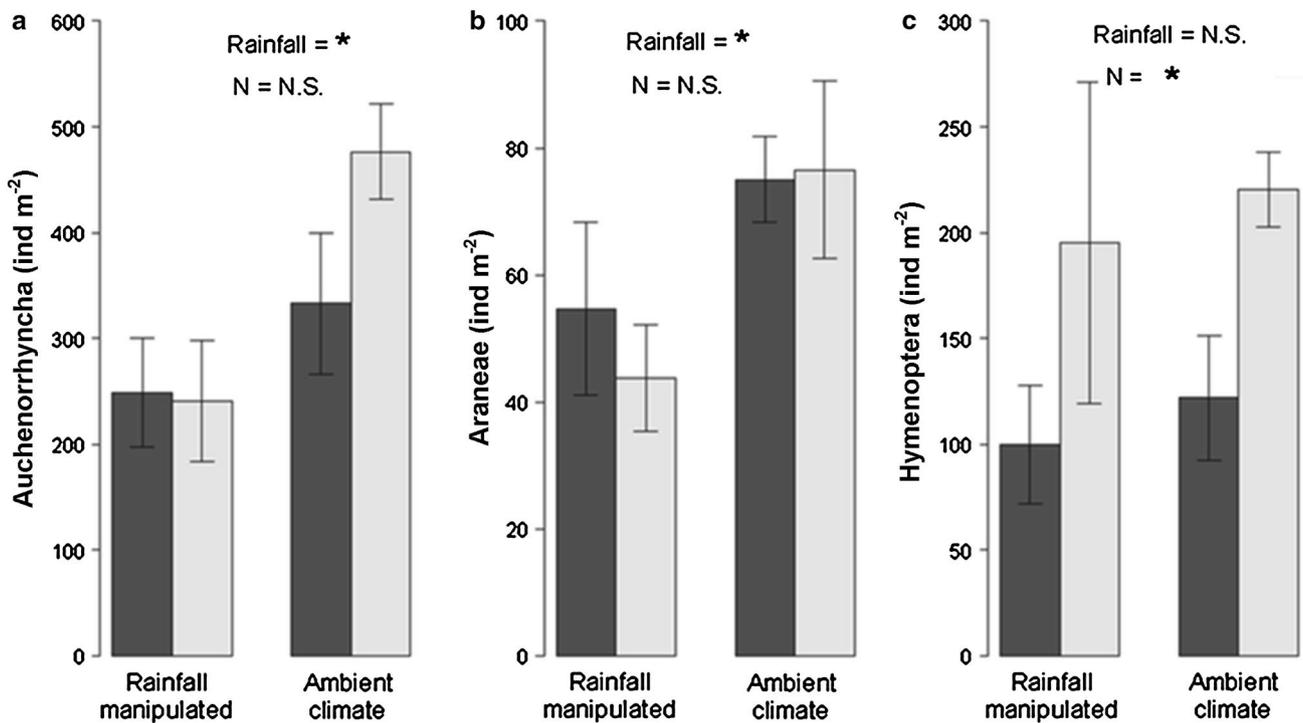
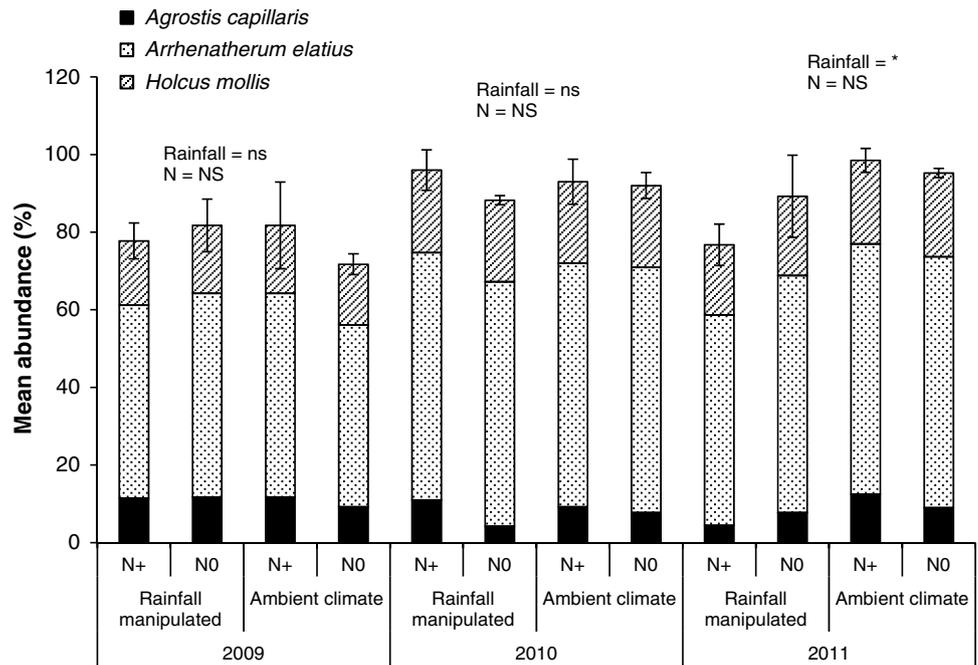


Fig. 4 Density of individuals (*ind*) of **a** Auchenorrhyncha, **b** Araneae and **c** Hymenoptera across all rainfall-manipulated, ambient climate, nitrogen addition (dark bars) and ambient nitrogen (light bars)

treatments. Values are the means of four replicates (\pm SE). Asterisk $P < 0.05$ and NS not significant ($P > 0.05$)

Discussion

Most measures of plant productivity and the composition of plant and arthropod communities were not strongly affected

by rainfall manipulation and N enrichment, either individually or in combination, in the first 2 years of the experiment. It should be noted that low replication in our study meant that relatively large effect sizes were frequently

not significant. However, many of the parameters that we measured in rainfall-manipulated plots displayed increasing effect sizes and declining P values over the 3-year period (e.g. vegetation height, vegetation cover and arthropod abundance). In the case of vegetation height, used as a proxy for above-ground biomass, the P value approached significance in year 3, whilst the effect size increased substantially—indicating a decline in vegetation height in rainfall-manipulated plots. This coincided with statistically significant destructive biomass harvests in year 3. Together, these results indicate that above-ground biomass production remained largely unchanged in years 1 and 2, but declined in rainfall-manipulated plots in year 3 when rainfall levels were lower than average. The general resistance of our system contrasts with rapid (<2 years) plant productivity declines and changes to plant species composition in response to a more severe short-term drought (0 % of rainfall re-applied in June and August) that was observed in another successional grassland in the same region (Grime et al. 2000). Our results are more in line with those from short-grass steppe, where the plant community remained unchanged in the first 3 years of a 50 % spring and summer rainfall reduction. This was attributed to the drought resistance of the dominant species, *Bouteloua gracilis*, whose shallow but dense root system is well suited to the rapid uptake of rainfall pulses (Evans et al. 2011).

Another mechanism that confers plant community resistance during the early stages of drought is higher investment of plant resources below ground to enable greater access to limited water supplies (Kalapos et al. 1996). The increase in root growth in the second year in rainfall-manipulated plots was large, thus providing some evidence for this mechanism, but was not statistically significant. Several other individual and community level changes may have also contributed to resistance to change. These include morphological changes associated with greater water use efficiency, succession to plants with a conservative growth strategy and elevated water storage within roots (Grime et al. 2000), though there is little evidence for these operating in our study. Particularly high rainfall in the first winter may also have promoted ecosystem resistance to summer rainfall reductions in the second year by enhancing deeper water reserves. This may explain the lack of change in soil moisture at the 0–15 cm depth, despite strong reductions in rainfall, as deep water reserves allow for recharge from lower layers during dry periods (Fry et al. 2014b).

Despite a lack of rainfall treatment effects in the first 2 years, a substantial decline in aboveground plant biomass (–28 %) was seen in the rainfall-manipulated plots in the third year of the experiment providing support for hypothesis one. Reduced plant productivity in response to long-term reductions in rainfall have been observed in many experiments across Europe and North America (Tilman

and Haddi 1992; Laporte et al. 2002; Morecroft et al. 2004; Harper et al. 2005; Yahdjian and Sala 2006; Evans et al. 2011), with relatively few displaying long-term resistance to rainfall reductions (e.g. Grime et al. 2000; Jentsch et al. 2011). In 2011, there was a generally dry spring and differences in the rainfall received by rainfall-manipulated and ambient climate plots in the summer were also strong, because most rainfall events fell under our 20-mm reapplication threshold. This drier-than-usual spring may have overwhelmed any buffering effect of winter enhancement of soil water reserves in rainfall-manipulated plots at this time, resulting in more marked changes to the ecosystem.

Reductions in total plant biomass in rainfall-manipulated plots were associated with changes in the relative abundances of different plant functional types. There were no significant differences between treatments for grass or forb cover throughout the experiment. However, reduced grass cover in rainfall-manipulated plots approached significance in year 3 and the effect size increased substantially. Again, this result, combined with the statistically significant decline in grass biomass in rainfall-manipulated plots detected by destructive harvests, indicates that plant compositional changes occurred in the final year only. The decline in grass biomass in the third year was slower than in a limestone grassland experiencing a more extreme summer drought (10 % of ambient rainfall reappplied) (Morecroft et al. 2004), and in a lowland prairie experiencing a 50 % lengthening of inter-rainfall dry intervals, but unaltered total rainfall volume (Fay et al. 2003). In the current study, the decline in overall grass biomass was associated with declines in the three dominant grass species: *Agrostis capillaris*, *Arrhenatherum elatius* and *Holcus mollis*, all three of which are typically found growing on moist soils (Hill et al. 1999). In contrast, there were no effects of rainfall manipulation on forbs. This may be due to regeneration from seed counteracting drought-induced mortality, a mechanism that has been observed in similar studies in temperate European grasslands (Jentsch et al. 2011). Elsewhere, grassland ecosystems have become dominated by drought-adapted C4 grasses at the expense of C3 grasses in response to reduced rainfall (Knapp and Smith 2001). In the absence of C4 species, as here, it may be that forbs are best able to capitalise on the decline of C3 grasses in response to reduced rainfall, as they are typically deeper rooted and thus able to access deeper, less temporally variable soil water (Schenk and Jackson 2002).

The persistence of the rainfall effects observed here remain uncertain, and there are insufficient data to predict whether rainfall-manipulated plots will recover in subsequent years. Some studies have shown that the productivity of drought-affected grasslands can recover in the following summer if rainfall recharges deeper water reserves (e.g. Jentsch et al. 2011). Our grassland, which appeared to

display this property in the first 2 years of our study, may possess such resilience. In such cases, ecosystem structure may be largely unaltered by periodic drought, and may be predictable from annual weather conditions alone.

Whilst there were some strong plant community responses to rainfall manipulation, there was no measurable effect of N enrichment on plant community composition or biomass accumulation throughout the 3-year experiment. This means that hypothesis two was not supported by our data, despite large increases in nitrification rates in N⁺ plots within the first year. Background N deposition inputs are fairly high at this site, and are already within the critical N load range for mesotrophic grasslands. The field site may therefore have been already N-saturated from the start of the experiment. In addition, the soils which form on the Bagshot Beds have a low P sorption capacity and as a result are P-limited (Manning et al. 2006). Consistent with this, concentrations of extractable P were shown to be low in a previous study conducted at this site (mean 3.56 mg kg⁻¹; Fry et al. 2013). The observed lack of response to N addition is similar to that found in N-enrichment studies on calcareous grasslands, which are typically P-limited (Wilson et al. 1995; Carroll et al. 2003). In most cases, N enrichment is associated with biomass increases and species losses, with rapid N acquisition and growth in a small number of nitrophilic species fostering competitive advantages over subordinate species (Bobbink et al. 2010; Manning 2012). The absence of this response in our study demonstrates that this general relationship is not universal, and that sites that already are N-saturated and/or P-limited are unlikely to respond in this way.

Limitation by nutrients other than N or P (Tsialtas et al. 2001) or strong microbial immobilisation of N inputs could also limit plant community responses to N addition and preclude any interactions between N and changing soil water status (Bardgett et al. 2003), though nitrification increases in N⁺ plots suggests that the latter explanation is unlikely. A third explanation for the lack of response to N could be leaching of the added N. This is also unlikely, since N did not accumulate in rainfall-manipulated plots and a previous study in adjacent plots found that leaching losses of N were low at this site (Fry et al. 2013). The lack of a main effect of N, and also an interaction between N and rainfall, meant that hypothesis three was also not supported by our data. This may have been caused by generally dry weather during the study period. This meant that even ambient plots received low levels of rainfall, perhaps resulting in universally low availability of N in solution (in 2010, 633 mm of annual rainfall was 17 % lower than the mean from the previous 4 years). Although this grassland system appears relatively unresponsive to increased N inputs and the combination of N inputs and rainfall change, care must be taken in extrapolating these results to other

grassland types where soil nutrient availability differs and the plant species present are adapted to different nutrient and water regimes (Critchley et al. 2002).

Decreases in plant-feeding Auchenorrhyncha in rainfall-manipulated plots are likely to have been caused by reduced plant biomass and the loss of cell turgor pressure that inhibits feeding under summer drought conditions (Huberty and Denno 2004). All other plant-feeding groups were unaffected by our treatments, and so increased herbivory is unlikely to have contributed to the decline in plant biomass that was recorded under rainfall-manipulated conditions. A change in arthropod community composition in rainfall-manipulated plots is in line with the findings of Boyer et al. (2003), who demonstrated that changes in water and N availability caused bottom-up effects on arthropod community composition in manipulated grasslands, particularly among sap-feeders and grazers. The decline in the abundance of Araneae in rainfall-manipulated plots suggests that the bottom-up effects of reduced prey availability and/or lower plant biomass (via effects on hunting/shelter availability) may have driven these changes, and it is unlikely therefore that top-down processes were responsible for the decline in Auchenorrhyncha. Less common plant-feeding groups such as Heteroptera and Isopoda did not respond to treatments, suggesting that neither food nor habitat were limiting when plant biomass declined (Isopoda are mainly litter feeders). A lack of response by common detritus-feeding Collembola may have also been because food availability was not limiting or may have been due to drought resistance of some groups (Lindberg and Bengtsson 2005). Increased leaf palatability (through elevated N and P contents) can have positive effects on herbivorous arthropod populations following reduced rainfall (Wright et al. 2001) or nitrogen addition (Throop and Lerdau 2004). Although leaf C:N:P ratios may have been modified in rainfall-manipulated and N-enriched plots, there are insufficient data available to elucidate the role of foliar chemistry in invertebrate community responses in this study.

It should be noted that there are difficulties in estimating and interpreting treatment effects on mobile groups, such as Coleoptera, Diptera and Hymenoptera, at the population and community level when using small and spatially aggregated plots. There were a greater number of Hymenoptera in rainfall-manipulated plots in 2010 and in N-enriched plots in 2011, compared with controls. These increased abundances may have been driven by changes to flowering times (Burkle and Irwin 2010) or the availability of nectar and pollen (Tooker and Hanks 2000). However, due to the mobile nature of Hymenoptera, these may also have been behavioural or transient, as opposed to population level, effects. Other predatory or omnivorous groups, such as the Coleoptera and Diptera, did not respond to treatments throughout the study, and the highly mobile nature of these

groups means that they can relocate to more suitable foraging areas. Despite these limitations, results like these can provide clues to changes in invertebrate behaviour and/or abundance that may occur in response to environmental changes. Overall, the changes to arthropod community composition that were recorded in the third year—demonstrated by a reduction in plant-feeding Auchenorrhyncha and predatory Araneae in rainfall-manipulated plots and an increase in Hymenoptera in nitrogen-enriched plots—may have important implications for biodiversity in mixed landscapes, where the habitat matrix consists of semi-natural grasslands and fertilised arable crops, and where grassland arthropods utilise both of these habitats (Ward and Masters 2007).

Other experiments involving multiple drivers of global change (N, CO₂, rainfall and warming) in temperate grasslands have identified N as the key driver of plant productivity increases (Dukes et al. 2005) and CO₂ and N as being important drivers of plant diversity declines (Zavaleta et al. 2003). These findings contrast with those presented here, which are likely to be due to differences in experimental design and initial ecosystem properties. Rainfall was increased in these earlier studies, rather than decreased, and study sites were dominated by plant species adapted to a much drier Mediterranean climate where N availability is limited. Assessing the cause of such differences by conducting standardised experiments, at multiple sites and over long periods, may help to identify the generalities and modifiers of these relationships (e.g. Fraser et al. 2013).

Conclusions

The vast majority of global change ecology research has looked at single drivers but in reality drivers will operate simultaneously. This study helps to address this knowledge gap by providing evidence of short-term (<2 years) resistance of plant and arthropod communities in a successional grassland ecosystem in response to predicted changes to local rainfall (IPCC 2007; Murphy et al. 2009) and N enrichment. However, the observed resistance to rainfall manipulation was relatively short-lived, and above-ground biomass decreased in the longer term (>2 years), with plant biomass declines driven by a reduction in cover of the dominant three grass species. Rainfall manipulation also caused changes to other trophic levels by the third year of the experiment, reducing the number of Araneae and Auchenorrhyncha within associated invertebrate communities. N enrichment and rainfall manipulation treatments applied together did not result in a change to plant community composition or biomass throughout the 3-year experiment. This indicates that the expected N-driven plant community changes or changes driven by interactions between N and

reduced rainfall are not universal. This finding adds to evidence that multiple concurrent global change drivers will not always have strongly interacting effects. The generality of these findings is unknown, since the characteristics of grasslands that drive community resistance and susceptibility to global changes are not fully quantified. Nevertheless, if these findings are a general phenomenon, our results may have important implications for predictions of the future delivery of a range of ecosystem services currently provided by grasslands. For example, changes to biomass production may impact upon provision of livestock forage and the removal of anthropogenic greenhouse gas emissions, and changes to predator and herbivore abundances may alter pest abundance in the wider agricultural landscape.

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