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Grassland biodiversity restoration increases resistance of carbon fluxes to drought

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Running headline: Grassland restoration increases resistance to drought

Abstract

1. Evidence suggests that the restoration of plant diversity in grasslands not only brings benefits for biodiversity conservation, but also the delivery of ecosystem services. While biodiversity-function experiments show that greater plant diversity increases resistance of plant productivity to climate extremes, it is not known whether real-world management options for grassland restoration likewise stabilise ecosystem responses to extreme climate events.
2. We used a long-term (23 year) field experiment in northern England to test the hypothesis that management aimed at biodiversity restoration increases the resistance and recovery of ecosystem carbon (C) fluxes to short-term summer drought. This was tested by measuring plant, soil and microbial responses to a simulated drought in experimental grassland plots where fertiliser application and seed addition have been managed to enhance plant species diversity.
3. The cessation of fertiliser application brought about small increases in plant species richness. Additionally, cessation of fertiliser application reduced overall plant productivity and promoted hemi-parasitic plants at the expense of grasses and forbs.
4. Resistance of CO₂ fluxes to drought, measured as ecosystem respiration, was greater in non-fertilised plots, as lower plant biomass reduced water demand, likely aided by proportionally more hemi-parasitic plants further reducing plant biomass. Additionally, legumes increased under drought, thereby contributing to overall resistance of plant productivity.
5. Recovery of soil microbial C and nitrogen was more rapid after rewetting than soil microbial community composition, irrespective of restoration treatment, suggesting high resilience of soil microbial communities to drought.

6. **Synthesis and applications.** This study shows that while grassland diversity restoration management increases the resistance of carbon fluxes to drought, it also reduces agricultural yields, revealing a trade-off for land managers. Furthermore legumes, promoted through long-term restoration treatments, can help to maintain plant community productivity under drought by increasing their biomass. As such, grassland management strategies not only have consequences for ecosystem processes, but also the capacity to withstand extreme weather events.

Keywords: biodiversity, carbon cycling, drought, fertiliser, grassland restoration, seed addition, soil microbial community.

Introduction

The restoration of plant diversity in grasslands, and the management practices required to bring it about, has been a major focus of research (Smith *et al.* 2000; Carter & Blair 2012). In addition to increasing plant diversity, grassland restoration can bring benefits for ecosystem services such as increasing soil carbon (C) accumulation (De Deyn *et al.* 2011), plant productivity (Bullock *et al.* 2001) and nutrient retention (Maron & Jefferies 2001). While biodiversity-function experiments show that greater plant diversity can increase the resistance of plant productivity to climate extremes (Isbell *et al.* 2015), it is not known whether real-world management options for the restoration of grassland plant diversity likewise stabilise ecosystem responses to extreme climate events. One such extreme climate event is drought, which is predicted to become more frequent and intense under climate change (IPCC 2013), with potential to disrupt C and nitrogen (N) cycling in grasslands (Harper *et al.* 2005).

In the face of environmental perturbations such as drought, management is required to maintain the stability of ecosystem functions and services (Oliver *et al.* 2015; Donohue *et al.* 2016). Although experimental studies show that greater species richness can increase the resistance of plant productivity to drought (Isbell *et al.* 2015), it is not known whether real-world grassland biodiversity restoration has a similar effect, and whether impacts of drought on plant productivity are less in restored than unrestored, species-poor grassland (Carter & Blair 2012). However, plant species introduced through restoration are likely to have differing physiological adaptations to survive drought than those already present on unrestored grassland (Hoekstra *et al.* 2014). For example, as drought can limit N availability to grassland plants, in part through reduced N mobility in soil, the presence of legume species (with N-fixing rhizobial associations) may increase community resistant to drought (Hofer *et al.* 2016). Although research has revealed restoration treatments which successfully increase plant diversity, it remains to be seen whether there are also benefits of successful biodiversity restoration treatments in terms of buffering grassland responses to drought.

Although drought can have direct effects on soil microbial communities, studies show that the response of plants to drought can also bring about indirect effects belowground (Bloor & Bardgett 2012). Specific groups of microbes (e.g. fungi, bacteria and archaea) respond differently to drought (Schimel, Balser & Wallenstein 2007), influencing the flow of C from plant roots into the soil (Fuchslueger *et al.* 2014). Further, recent studies show that grassland management can alter the resistance and resilience of C cycling processes to drought by changing patterns of plant C allocation and C transfer to the microbial community (Karlowsky *et al.* 2018). This suggests grassland biodiversity restoration could likewise alter the resistance and resilience of soil microbial processes to drought, for example through the promotion of drought-tolerant groups such as fungi or gram-positive bacteria (Smith *et al.* 2008), which may in turn promote soil C and N retention (De Vries *et al.* 2012b). However, it remains unclear whether changes in the soil microbial community associated with biodiversity restoration, will in turn affect how grassland C and N cycles respond to drought.

The goal of this study was to test whether long-term management treatments used to restore grassland plant diversity confer greater resistance and recovery of C and N cycling processes and microbial communities to drought. This was achieved using a 23-year grassland biodiversity restoration experiment at Colt Park Meadows, northern England, which has successfully brought about increased plant diversity through a combination of mixed species plant seed addition and cessation of annual inorganic fertiliser application, alongside shifts in the abundance of plant functional groups (Smith *et al.* 2000, 2008; De Deyn *et al.* 2011). Additionally, the combined treatments of cessation of fertiliser application and seed addition, has been associated with concurrent changes in the soil microbial community, in particular an increase in the abundance of fungi relative to bacteria (Smith *et al.* 2008). Such changes in microbial community composition have been linked to greater nutrient retention (Bardgett & McAlister 1999; De Vries *et al.* 2012a) and an increase in soil C accumulation when combined with legume addition (De Deyn *et al.* 2011).

We used rain-out shelters on selected treatments to test the hypothesis that the resistance and recovery of C and N cycling to summer drought is enhanced by restored plant diversity. Specifically, we hypothesised that: (H1) drought would reduce plant productivity, ecosystem respiration, and microbial biomass and have a greater negative effect on soil bacteria than fungi; and (H2) the resistance and recovery of C and N cycling processes to drought would be greater in treatments subject to long-term fertiliser cessation and seed addition. This treatment combination has been associated with lower plant productivity, which reduces water demand, and a greater abundance of fungi relative to bacteria in soil, which has been shown to buffer effects of drought events on soil processes (De Vries *et al.* 2012b).

Materials and methods

Experimental system

The study was conducted on selected plots of a long-term (23-year) grassland diversity restoration experiment at Colt Park meadows, Ingleborough National Nature Reserve, northern England (latitude 54°12'N, longitude 2°21'W; Bardgett & McAlister 1999; Smith *et al.* 2000; De Deyn *et al.* 2011).

The experiment was set up in 1990 on agriculturally improved *Lolium perenne*-*Cynosorus cristatus* grassland on brown earth soils over limestone bedrock, in order to identify optimal management strategies for the restoration of botanical diversity (Smith *et al.* 2000). Onto this long term restoration experiment, we superimposed short-term drought treatments on a subset of 12 plots (3m x 3m) in 3 replicate blocks from factorial treatment combinations, namely cessation or continued application of fertiliser and with or without seed addition. These treatments were selected because the combination of seed addition and cessation of inorganic fertiliser addition has resulted in the greatest increase in plant diversity (Smith *et al.* 2008; De Deyn *et al.* 2011).

Before the long-term grassland restoration was initiated the meadow received regular additions of inorganic fertiliser (Smith *et al.* 2000). The fertiliser cessation treatment started in 1990 with the alternate treatment being continued fertiliser application (NPK 20:10:10; 25kg N ha⁻¹) which has since been applied to plots by hand annually in spring (21 May in 2013), except in 2009 and 2010. Seed addition treatment also started in 1990 with seed of 19 species coming from locally collected and commercially bought seed. Since 1999 all plots have been cut for hay between mid-July and August (16 July in 2013), and then grazed by sheep and cattle until hay production in May (Smith *et al.* 2000, 2003, 2008; De Deyn *et al.* 2011). The year prior to establishing the drought experiment, species richness across 4m² was greatest in the fertiliser cessation treatment (26.5±1.4) and lowest with continued fertiliser application (22.7±1.6; LRT=4.70, d.f.=1,5, P=0.030).

Drought treatments

To investigate the effect of drought on C and N cycling in grasslands, we set up 3 levels of the drought treatment in each of the 12 long-term experimental plots in June 2013 (Fig 1). The three treatments were: ambient (no rain-out shelter), drought (rain-out shelter) and roofed control (rain-out shelter with holes). Rain-out shelters were open sided, constructed of transparent corrugated PVC, 0.8mm thick (Corolux, UK). Shelters were 90cm x 105cm with a height of 38cm-63cm, giving a sloped roof of 16 degrees. Roofed control shelters were identical to those used in the drought treatment, except they contained holes to allow rainfall to reach the plot and were used to assess any artefacts of having rain-out shelters in place. The rain-out shelters were in place from 5 June-10 July 2013 (35 days) to match the length of 100-year drought events. For this, long-term precipitation data from Malham tarn was fitted with Gumbel I distribution for consecutive days with <1mm precipitation during the primary growth period (April-September), following Bloor & Bardgett (2012). The effect of rain-out shelters on soil moisture was assessed using a ThetaProbe soil moisture meter (Delta-T, UK), and temperature was measured using Hobo Pendant temperature loggers (Onset, USA) at 5cm depth for soil and 5cm above soil surface for air temperature in ambient, control shelter and drought plots. Measurements of soil DOC and DON, soil microbial community and vegetation C and N content were only made on the ambient and drought treatment and not the roofed control treatment.

Plant community

Aboveground plant biomass was harvested from all plots on 10 July, after the end of the drought treatment. The plant biomass was dried at 60°C for 48 hours and split by hand into plant functional types (PFT): grasses, forbs, legumes and hemi-parasitic plants. After ball milling, C and N content for each PFT was measured on a Tru-spec CN analyser (Leco, St. Joseph, MI, USA). Vascular plant species surveys were carried out between 29 June and 4 July 2013 on the central 706cm² of each plot.

CO₂ flux measurements

Net ecosystem exchange (NEE) and ecosystem respiration were measured using static chambers following Ward *et al.* (2013), linked to an infra-red gas analyser (EGM4, PP Systems, UK), as used by De Deyn *et al.* (2011). Two minute headspace closures were used for NEE (transparent chambers) and ecosystem respiration (opaque chambers) between 10:15 and 16:30 hrs alongside measurements of photosynthetically active radiation (PAR), soil and air temperature. Two measurements were made before the drought (17, 24 May), six during the experimental drought (13, 20, 24, 27 June; 5, 8 July 2013) and five after shelter removal (19, 26, 30 July; 7, 16 August 2013).

Soil microbial community

Soil was sampled at four time-points with three cores (2.4cm diameter, 10cm depth) taken from each subplot, bulked together and sieved (2mm). Sampling dates were 5 June (before drought), 10 July (during drought), 16 August (3 weeks after rewetting) and 4 November (3 months after rewetting). Before the drought treatment was imposed, samples were only taken from the 12 main plots to determine treatment effects of seed addition and fertiliser cessation. To quantify belowground biomass, roots were removed from soil cores sampled during the drought with roots sieved, washed and dried at 60°C for 48 hours before weighing.

The effect of grassland restoration and drought on bacterial and fungal community composition was assessed using the terminal restriction fragment length polymorphism (T-RFLP) method as detailed by Plassart *et al.* (2012). Genomic DNA was extracted from soil samples using the PowerSoil kit (MoBio, Carlsbad, US) and amplified using primers for bacterial 16S rRNA and fungal ITS genetic markers. In addition, broad-scale changes in soil microbial community composition were assessed by phospholipid fatty acid analysis (PLFA). Briefly, PLFA's were extracted from freeze-dried soil using a modified Bligh-Dyer extraction and separated from other lipids using aminopropyl solid phase extraction cartridge (Phenomenex, US; White *et al.* 1979). Gas chromatography was carried out on an Agilent 6890GC with fused silica capillary column (Agilent, US). Biomarkers were used for bacteria

(i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy-17:0, 18:1 ω 7 and cy-19:0) and saprotrophic fungi (18:2 ω 6,9) (Bardgett, Hobbs & Frostegard 1996; Smith *et al.* 2008).

Microbial biomass C and N were measured on 5g fresh soil subsamples using the chloroform fumigation-incubation method (Brookes *et al.* 1985) in soil cores taken before, during and after (August) the drought. One subsample was fumigated with chloroform for 16 hours before extraction with 25mL 0.5M K₂SO₄. The resulting filtrate was analysed for microbial C using a TOC analyser (5000A, Shimadzu, Milton Keynes, UK). For microbial N, filtrate was oxidised with K₂S₂O₈ before colorimetric analysis on an autoanalyser (Bran and Luebbe, Northampton, UK). Adjustment factors were applied, using K_c=0.35 for microbial C and k_n=0.54 for microbial N (Bloor & Bardgett 2012). For soil samples during and after (August) the drought, DOC and DON were extracted from 5g subsamples using 35mL of water with DOC analysed on a TOC analyser (5000A, Shimadzu, Milton Keynes, UK) and DON extract oxidised with K₂S₂O₈ before colorimetric analysis (Bran and Luebbe, Northampton, UK).

Statistical analysis

To investigate responses of plant and soil measurements to drought, we used percentage change caused by drought and rewetting as indices of resistance and recovery, as widely used in previous studies (reviewed in Orwin & Wardle 2004). Specifically resistance and recovery were calculated as $(P_0 - C_0) / C_0 \cdot 100$, where P₀ is the drought treatment response and C₀ is the control response. Where data were available, resistance and recovery were calculated using both the ambient and control shelter treatments as C₀ to allow the effect of the rain-shelter to be investigated.

Linear mixed effects models (LME) were used to investigate plant and soil microbial responses to seed addition, fertiliser and drought treatments. For each LME model the fixed effects were seed, fertiliser, drought and all interactions. The random effect was split-plot nested within block to take account of the experimental split-plot design. Where data included multiple measurements from a single plot, plot ID was added as an additional nested random effect. Assumptions of normality and

equal variances were checked graphically and where necessary response variables were either logged or used *varIdent* weight functions to improve model fit [e.g. `varIdent(form=~1|fertiliser)`], following Zuur *et al.* (2009). We determined the significance of fixed effects by comparing models with and without the factor of interest using likelihood ratio tests (LRT). To investigate changes in soil microbial community structure, the relative abundance of T-RF peaks was assessed using between-sample Bray-Curtis dissimilarities and non-metric multidimensional scaling with permutational multivariate analysis of variance. All statistical analysis was carried out in the R programming language 3.3.1 (R Core Development Team, 2016) using the *nlme* package for mixed effect models (Pinheiro *et al.* 2013).

Results

Rain shelter effects on soil moisture and temperature

The drought treatment excluded 180.8mm of rainfall over 35 days with significant rewetting occurring in late July, more than a week after rain-out shelters were removed (Fig 1b). The treatment intercepted rainfall equivalent to 7.5% of average annual precipitation and reduced soil moisture from 58.8% to 33.3%, while the roofed control shelter also intercepted some rainfall and reduced soil moisture from 58.8% to 49.8% (Fig 1a, LRT=113.55, d.f.=2,10, $P<0.0001$). Additionally, fertiliser application reduced soil moisture by 3% relative to cessation of fertiliser (LRT=5.68, d.f.=1,11, $P=0.017$). Two nearby sites with long-term precipitation datasets suggest that 100-year extreme drought events in the primary growing season equate to 27 and 34 days with <1mm rainfall (Bloor & Bardgett 2012), similar to the 35 days rain-out shelters were in place in this study. No effects of rain-out shelters were detected on mean air temperature (LRT=2.45, d.f.=2,6, $P=0.293$) or soil temperature (LRT=1.72, d.f.=2,7, $P=0.424$).

Plant community

Cessation of fertiliser use brought about an average 1.2 species increase in plant species richness (LRT=6.46, d.f.=1,10, $P=0.011$), while seed addition increased species richness, but only in the ambient treatment which had no rain shelter (Fig 2a, drought x seed: LRT=8.59, d.f.=2,13, $P=0.014$).

Total aboveground biomass, harvested in July, was 49.4% lower in non-fertilised compared to fertilised plots (LRT=18.45, d.f.=1,10, $P<0.0001$), while seed addition had no effect (Fig 2b, LRT=1.36, d.f.=1,10, $P=0.244$). For specific plant functional types, grass and forb biomass was respectively 57.2% and 35.2% lower in non-fertilised relative to fertilised plots (Fig 3, Grass: LRT=19.02, d.f.=1,10, $P<0.0001$; Forb: LRT=9.43, d.f.=1,11, $P=0.002$), while hemi-parasitic plant biomass was nearly double (Fig 3d, LRT=3.94, d.f.=1,10, $P=0.047$). As a consequence, the proportion of plant functional groups shifted in non-fertilised plots, with proportionally less grass biomass and more legume and hemi-parasitic plant biomass (Fig S1).

Drought, superimposed across the long-term grassland restoration treatments, did not reduce total aboveground (Fig 2a, LRT=2.50, d.f.=2,9, $P=0.286$) or root biomass (Fig 3a, LRT=2.93, d.f.=2,9, $P=0.231$). However, legume biomass increased under drought (Fig 2c, LRT=8.00, d.f.=2,11, $P=0.018$), whereas for grasses drought had no impact, although forb biomass was marginally increased by drought but only in plots with seed addition (Fig 3). Additionally, the hemi-parasitic plant species *R. minor* was more abundant in plots without than with seed addition, but primarily so under drought conditions (Fig 3e, drought x seed: LRT=6.43, d.f.=2,13, $P=0.040$). Furthermore, in grasslands with seed addition the resistance of N content in shoot biomass was increased across all forbs, grasses and legumes (Fig 4a, LRT=5.52, d.f.=1,12, $P=0.019$).

CO₂ fluxes

Ecosystem respiration increased with soil temperature (LRT=12.57, d.f.=1,77, $P=0.0004$), while continued fertiliser application also increased ecosystem respiration, with generally larger increases later in the growing season (Fig S2, date x fertiliser: LRT=11.56, d.f.=5,52, $P=0.041$). Furthermore,

grasslands with continued fertiliser application also had the greatest reduction in ecosystem respiration under drought compared with ambient and control shelter treatments (Fertiliser x drought: LRT=6.83, d.f.=2,55, $P=0.033$), although the impact of drought was greatest in mid-June (Fig S2, date x drought: LRT=20.52, d.f.=10,47, $P=0.025$). As such, the reduction in ecosystem respiration correlated negatively with aboveground plant biomass compared to both ambient and control shelter treatments (Fig 6). Consequently, resistance of ecosystem respiration to drought, expressed as percentage change, was reduced by continued fertiliser application relative to ambient plots with no rain-shelter (LRT=3.86, d.f.=1,16, $P=0.049$) and control shelters plots (Fig 5, LRT=5.41, d.f.=1,16, $P=0.020$).

After the hay cut, ecosystem respiration increased over time, particularly in fertilised plots (Date x fertiliser: LRT=18.22, d.f.=4,45, $P=0.001$) and in those previously exposed to drought, although the increase occurred more quickly with seed addition (Fig S2, date x seed x drought: LRT=16.48, d.f.=8,57, $P=0.036$). Recovery of ecosystem respiration, expressed as percentage increase, also suggested recovery was initially lower in grasslands with seed addition, but increased two to three weeks after rewetting in early August, with a similar pattern when using either ambient treatment (Seed x date: LRT=12.99, d.f.=4,19, $P=0.011$) or control shelter as reference treatment (Fig 5, seed x date: LRT=11.03, d.f.=4,19, $P=0.026$).

NEE increased with PAR (LRT=85.09, d.f.=1,84, $P<0.0001$), soil temperature (LRT=4.88, d.f.=1,84, $P=0.027$) and fertiliser application, although this differed through the growing season (Fig S3, fertiliser x date: LRT=21.06, d.f.=5,59, $P=0.0008$). Additionally, NEE varied slightly across drought treatments depending on sampling date (Fig S3, date x drought: LRT=31.52, d.f.=10,54, $P=0.0005$). Resistance of NEE to drought increased with fertiliser application on particular sampling dates, calculated as percentage change relative to ambient plots with no rain-shelter (Fig S4a, fertiliser x date: LRT=13.74, d.f.=5,22, $P=0.017$) and control shelters plots (Fig S4c, fertiliser x date: LRT=13.65, d.f.=5,22, $P=0.018$).

After natural rewetting, NEE increased with PAR (LRT=30.50, d.f.=1,66, $P<0.0001$) and increased over time, but varied with fertiliser cessation and seed addition (Fig S3, date x fertiliser x seed: LRT=11.04, d.f.=4,58, $P=0.026$). Recovery of NEE, expressed as percentage change after rewetting, varied across sampling dates (Fig S4b, LRT=12.21, d.f.=4,11, $P=0.016$), while when using the control shelter as the reference treatment, recovery was marginally slower in communities with seed addition, but only when combined with fertiliser application (Fig S4d, fertiliser x seed: LRT=4.12, d.f.=1,23, $P=0.042$).

Soil properties and microbial community

Microbial biomass C and N were partially reduced by drought (Fig S5), with reduced resistance in grasslands with seed addition (Fig 4b, microbial biomass C: LRT=12.47, d.f.=1,5, $P=0.0004$; microbial biomass N: LRT=4.79, d.f.=1,6, $P=0.029$). Three weeks after the drought, both microbial biomass C and N showed full recovery to levels in non-droughted plots (Fig S5, 4c). In contrast to soil DOC, soil DON was partially reduced by drought (Fig S6), with reduced resistance in grasslands without seed addition (Fig 4b, LRT=4.71, d.f.=1,5, $P=0.030$). However after rewetting, recovery of DON was almost 4 fold greater with continued fertiliser application (Fig 4c, LRT=6.07, d.f.=1,7, $P=0.014$).

Bacterial PLFAs did not change in response to drought or rewetting (Fig 7a, S5), while bacterial community composition, assessed by T-RFLP, was altered by drought (Fig S3, $F=3.28$, d.f.=1,12, $P=0.010$), although after rewetting in August the community differed with seed addition (Fig S3, $F=3.04$, d.f.=1,14, $P=0.020$). In contrast, fungal PLFA generally increased under drought (Fig S7f, fertiliser x seed x drought: LRT=4.26, d.f.=1,11, $P=0.039$), although after rewetting recovery was slower in grasslands with seed addition and fertiliser application (Fig 7b) but with no difference in recovery in November (Fig 7b).

Discussion

The aim of this study was to investigate whether real-world management options for the restoration of grassland plant diversity brought about changes in the capacity for plant and soil microbial communities to resist and recover from drought. Differences in plant species richness across treatments were small, but this study suggests grasslands most resistant to drought are likely to be those with greater proportions of legumes and hemi-parasitic plants, but lower plant biomass. This indicates a trade-off for management between resistance to drought and agricultural yield. Overall, this study suggests that biodiversity restoration management through long-term fertiliser cessation and addition of key plant species can have positive effects for resistance to summer drought events.

In this study, drought was superimposed on a 23-year grassland restoration experiment in which seed addition and fertiliser cessation have brought about the greatest increase in plant diversity (Smith *et al.* 2003, 2008). Following previous studies, fertiliser cessation and seed addition have continued to bring about generally small increases in species richness, alongside shifts in plant functional groups from grasses to legumes and hemi-parasitic plants and an increase in soil fungi relative to bacteria (Smith *et al.* 2000, 2003, 2008).

The impact of drought on grasslands subject to biodiversity restoration management was tested using both a complete rain shelter and a control shelter with holes to test for any unanticipated effects of the shelter (Vogel *et al.* 2013). The control shelter reduced soil moisture by 9% making it difficult to separate unanticipated side-effects of the shelter from those caused by the reduced soil moisture. Even so, any unanticipated artefacts of the shelter were most likely to be seen in CO₂ fluxes, which were sensitive to PAR and soil temperature, and which can be impacted by shelters (Vogel *et al.* 2013). In contrast, the reduction in ecosystem respiration under drought was similar irrespective of whether it was relative to the ambient or control shelter treatment (Fig 6). This suggests that results are unlikely to be impacted by unanticipated artefacts of the rain shelter, with overall conclusions robust to

changing how drought data are included in statistical models (Table S2, S3). Therefore the subset of measurements that were not able to include the extra level of control are still likely to represent the effect of drought rather than the shelter.

Drought and plant community

Contrary to the first hypothesis (H1), plant productivity was resistant to drought, however, legume biomass increased under drought irrespective of grassland restoration treatment. The increase in legumes under drought was only significant when compared to ambient (no shelter), and not the control shelter. This suggests the reduction in soil moisture under the control shelter may have led to intermediate legume biomass, although an unanticipated effect of the rain-shelter cannot be ruled out. Although communities containing legumes can have greater reductions in productivity under drought (Pfisterer & Schmid 2002), legumes may be more resistant to drought, as by accessing atmospheric N they can avoid the N limitation imposed on plants by drought due to reduced mobility of nutrients (Hofer *et al.* 2016). Additionally, both the two most common legume species in this study, *T. repens* and *T. pratense*, can access water from lower soil depths during water scarcity (Grieu *et al.* 2001; Hoekstra *et al.* 2014). Under severe drought, an increase in legumes may offset reductions in productivity of other plant functional types under drought, thereby helping to maintain overall plant productivity. In this long-term study *T. pratense* was initially introduced through seed addition (Smith *et al.* 2000) and promoted by cessation of fertiliser applications (Smith *et al.* 2003), which suggests that through introducing and promoting legumes, grassland restoration may increase resistance of plant productivity to drought.

Drought and CO₂ fluxes

As hypothesised (H1), drought reduced ecosystem respiration by 12.3% in grasslands with fertiliser addition but with minimal impact in grasslands with cessation of fertiliser. Additionally, this result was robust to the use of either ambient or control shelter treatments to calculate resistance indices. The greater resistance of ecosystem respiration to drought was likely due to low productivity

grasslands being more resistant to drought, due to reduced water demand (Wang, Yu & Wang 2007). This suggests a potential trade-off between agricultural yields and resistance to drought. In this long-term experiment, hay yields reduced as *R. minor* became abundant 6 years after initial seed addition (Smith *et al.* 2000), suggesting hemi-parasitic plants may increase resistance of C cycling to drought through reducing plant productivity, in addition to enabling slow-growing species to establish (Joshi *et al.* 2000, Bardgett *et al.* 2006). These conclusions, however, will differ where grassland biodiversity restoration has been found to increase agricultural yields, for example in seeding experiments on ex-arable land (Bullock *et al.* 2001). This suggests there may be variability in the impact of grassland biodiversity restoration on community resistance to drought, primarily due to the impact of restoration on agricultural yield.

Drought and soil microbial community

Drought reduced microbial biomass C and N in plots with seed addition, suggesting the drought response was modulated by the plant community composition (Bloor & Bardgett 2012; De Vries *et al.* 2018). Additionally, the increase in fungal PLFAs under drought may point to a strong link between plants and fungi under drought (Fuchslueger *et al.* 2014), potentially due to plants accessing water through the arbuscular mycorrhiza hyphal networks (Karlowsky *et al.* 2017). Unanticipated artefacts of the rain shelter, however, cannot be ruled out, due to not having data on soil microbial communities from the control shelter treatment. Nevertheless, our study suggests that the increase in the abundance of soil fungi over two decades of biodiversity restoration has led to a soil microbial community that is resistant to drought.

The soil microbial community showed high resilience after rewetting, irrespective of grassland biodiversity restoration treatment. Three weeks after rewetting, microbial biomass C and N had fully recovered, while the effect of drought on microbial community structure persisted. Additionally a large post-rewetting increase in soil DON followed similar increases in soil N availability post drought (Roy *et al.* 2016). Recovery of the soil microbial community after drought can be variable, with different microbial groups responding differently (De Vries *et al.* 2012b; Fuchslueger *et al.*

2014; De Vries *et al.* 2018); however our data show that microbial biomass C and N can recover more quickly than microbial community structure and this high resilience is irrespective of grassland restoration treatment.

Climate change and grassland restoration

This study suggests that the changes in plant and soil microbial communities brought about by drought match those that aid restoration from species poor to species rich grassland. On this long-term experiment, restoration was benefitted through introducing and promoting the hemi-parasitic species *R. minor* and legumes, which were associated with higher abundance of fungi relative to bacteria in soil (Smith *et al.* 2003; 2008). Similarly, in this study, grasslands exposed to drought had increased legume biomass, fungal-to-bacterial ratio and, in a subset of treatments, *R. minor* cover. Further work is needed to assess if these shifts in one growing season have longer-term consequences for development of species rich communities. In contrast, on ex-arable grasslands, drought may impair restoration by promoting ruderal species at the expense of species typical of old grasslands (Morecroft *et al.* 2004). This study suggests, when restoring agriculturally improved species-poor grasslands the plants promoted under drought can match those targeted by restoration treatments.

Research shows that grassland restoration can increase soil C accumulation (De Deyn *et al.* 2011), nutrient retention (Maron & Jefferies 2001), and resistance of plant productivity and C fluxes to drought (Wang *et al.* 2007; this study). Yet these benefits were all found to be associated with lower productivity grasslands or cessation of fertiliser application. This suggests multiple trade-offs between broader multifunctionality and agricultural yield when restoring grassland botanical diversity. Grassland restoration is commonly supported by agri-environment schemes, however payments are often linked to set estimates of income forgone rather than for the public goods they deliver (Fraser 2009). In the context of grassland restoration, the actual income forgone will be greater where restoration leads to low yields which may make payments less attractive to the land owner, but confer additional benefits (e.g. Wang *et al.* 2007). In contrast, grassland restoration which increases

agricultural yields will have minimal actual income forgone (Bullock *et al.* 2007), and may appear more attractive. This suggests a need to disentangle the trade-offs between the benefits of grassland restoration to the public (e.g. C sequestration) and land manager (e.g. productivity resistant to drought), and to what extent this may change under a future climate.

Conclusions

This study set out to investigate if real-world grassland biodiversity restoration treatments influence plant, soil and microbial responses to extreme climate events. Although increases in plant species richness were small, this study suggests that grassland biodiversity restoration increases resistance of C fluxes to drought, but that this is associated with lower plant biomass. This presents a trade-off for management between resistance to drought and higher agricultural yields, and suggests Agri-environment schemes may need to account for such trade-offs when seeking to deliver public goods. Additionally, grassland biodiversity restoration aiming to increase resistance to drought should increase legumes and hemi-parasitic plants, both of which were also beneficial for restoration from species poor to species rich grassland over the previous two decades. Overall, our results show that grassland management strategies aimed at the restoration of biodiversity, not only have consequences for ecosystem processes, but also their capacity to withstand extreme weather events. This has implications for future grassland management given that extreme weather events are expected to increase in intensity and frequency with climate change.

Authors' contributions

The drought experiment was initiated and designed by AJC, SEW, NO and RDB. Data collection and analysis was carried out by AJC. All authors contributed to the writing and reviewing of the paper and approve publication.

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Data accessibility

Data available via the Environmental Information Data Centre <https://doi.org/10.5285/8a41b2a2-01d7-409e-adf5-fba3f3770f29> (Cole *et al.* 2019).

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Figure legends

Figure 1. Soil moisture (a) and daily precipitation (b) through the experiment. –F represents fertiliser cessation, +F represents continued fertiliser application. Central x-axis indicates soil sampling (circles) and hay cut (triangle). Vertical dashed lines show installation and removal of rain-out shelters.

Figure 2. Plant community responses to biodiversity restoration treatments and drought; (a) species richness; (b) shoot biomass; (c) legume biomass, for ambient (A), control shelter (cS) and drought (D) treatments. The significance of main effects and interactions indicated by ***= $P < 0.001$, **= $P < 0.01$, *= $P < 0.05$ and for panel (c) different letters represent significant differences between treatments.

Figure 3. Plant community responses to restoration treatments and drought; (a) root biomass; (b) grass biomass; (c) forb biomass; (d) hemi-parasitic plant biomass; and (e) *Rhinanthus minor* % cover, for ambient (A), control shelter (cS) and drought (D) treatments. The significance of main effects and interactions indicated by ***= $P < 0.0001$, **= $P < 0.001$, *= $P < 0.01$, = $P < 0.05$.

Figure 4. Resistance (a) vegetation N content to drought and resistance (b) and recovery (c) of soil microbial and organic C and N pools to drought. Assessed as percentage change where positive values show increases under drought or following re-wetting. –S represents no seed addition, +S represents seed addition, –F represents fertiliser cessation, +F represents continued fertiliser application. The significance of main effects and interactions indicated by ***= $P < 0.001$, **= $P < 0.01$, *= $P < 0.05$

Figure 5. The resistance and recovery of ecosystem respiration to drought using the ambient treatment as reference treatment (a-b) and the control shelter treatment as reference treatment (c-d).

The significance of main effects and interactions indicated by $*=P<0.05$.

Figure 6. Reduction in ecosystem respiration with drought averaged across sampling from 20 June to 5 July. Dashed horizontal line indicates the same ecosystem respiration in ambient and drought.

Figure 7. The resistance and recovery to drought of: (a) bacterial; and (b) fungal PLFAs, assessed as percentage change where positive values show increases under drought or following re-wetting. -S represents no seed addition, +S represents seed addition, -F represents fertiliser cessation, +F represents continued fertiliser application. The significance of main effects and interactions indicated by $*=P<0.05$.









