

1 **Grassland biodiversity restoration increases resistance of carbon fluxes to**  
2 **drought**

3 Andrew J. Cole<sup>1,2</sup>, Robert I. Griffiths<sup>3</sup>, Susan E. Ward<sup>2</sup>, Jeanette Whitaker<sup>1</sup>, Nicholas J. Ostle<sup>2</sup>, Richard  
4 D. Bardgett<sup>4</sup>

5 <sup>1</sup> Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster  
6 LA1 4AP, UK.

7 <sup>2</sup> Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK.

8 <sup>3</sup> Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford  
9 OX10 8BB, UK.

10 <sup>4</sup> School of Earth and Environmental Sciences, Michael Smith Building, The University of Manchester,  
11 Oxford Road, Manchester M13 9PT, UK.

12

13 Correspondence: Andrew J. Cole, Centre for Ecology & Hydrology, Lancaster Environment Centre,  
14 Library Avenue, Bailrigg, Lancaster LA1 4AP, UK ([andjcole@gmail.com](mailto:andjcole@gmail.com))

15

16 Running headline: Grassland restoration increases resistance to drought

17

18 **Abstract**

19 1. Evidence suggests that the restoration of plant diversity in grasslands not only brings benefits  
20 for biodiversity conservation, but also the delivery of ecosystem services. While biodiversity-  
21 function experiments show that greater plant diversity increases resistance of plant productivity  
22 to climate extremes, it is not known whether real-world management options for grassland  
23 restoration likewise stabilise ecosystem responses to extreme climate events.

- 24 2. We used a long-term (23 year) field experiment in northern England to test the hypothesis that  
25 management aimed at biodiversity restoration increases the resistance and recovery of  
26 ecosystem carbon (C) fluxes to short-term summer drought. This was tested by measuring plant,  
27 soil and microbial responses to a simulated drought in experimental grassland plots where  
28 fertiliser application and seed addition have been managed to enhance plant species diversity.
- 29 3. The cessation of fertiliser application brought about small increases in plant species richness.  
30 Additionally, cessation of fertiliser application reduced overall plant productivity and promoted  
31 hemi-parasitic plants at the expense of grasses and forbs.
- 32 4. Resistance of CO<sub>2</sub> fluxes to drought, measured as ecosystem respiration, was greater in non-  
33 fertilised plots, as lower plant biomass reduced water demand, likely aided by proportionally  
34 more hemi-parasitic plants further reducing plant biomass. Additionally, legumes increased  
35 under drought, thereby contributing to overall resistance of plant productivity.
- 36 5. Recovery of soil microbial C and nitrogen was more rapid after rewetting than soil microbial  
37 community composition, irrespective of restoration treatment, suggesting high resilience of soil  
38 microbial communities to drought.
- 39 6. ***Synthesis and applications.*** This study shows that while grassland diversity restoration  
40 management increases the resistance of carbon fluxes to drought, it also reduces agricultural  
41 yields, revealing a trade-off for land managers. Furthermore legumes, promoted through long-  
42 term restoration treatments, can help to maintain plant community productivity under drought  
43 by increasing their biomass. As such, grassland management strategies not only have  
44 consequences for ecosystem processes, but also the capacity to withstand extreme weather  
45 events.

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

## 48 **Introduction**

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

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

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

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

96 We used rain-out shelters on selected treatments to test the hypothesis that the resistance and recovery  
97 of C and N cycling to summer drought is enhanced by restored plant diversity. Specifically, we

98 hypothesised that: (H1) drought would reduce plant productivity, ecosystem respiration, and microbial  
99 biomass and have a greater negative effect on soil bacteria than fungi; and (H2) the resistance and  
100 recovery of C and N cycling processes to drought would be greater in treatments subject to long-term  
101 fertiliser cessation and seed addition. This treatment combination has been associated with lower plant  
102 productivity, which reduces water demand, and a greater abundance of fungi relative to bacteria in soil,  
103 which has been shown to buffer effects of drought events on soil processes (De Vries *et al.* 2012b).

## 104 **Materials and methods**

### 105 *Experimental system*

106 The study was conducted on selected plots of a long-term (23-year) grassland diversity restoration  
107 experiment at Colt Park meadows, Ingleborough National Nature Reserve, northern England (latitude  
108 54°12'N, longitude 2°21'W; Bardgett & McAlister 1999; Smith *et al.* 2000; De Deyn *et al.* 2011). The  
109 experiment was set up in 1990 on agriculturally improved *Lolium perenne*-*Cynosorus cristatus*  
110 grassland on brown earth soils over limestone bedrock, in order to identify optimal management  
111 strategies for the restoration of botanical diversity (Smith *et al.* 2000). Onto this long term restoration  
112 experiment, we superimposed short-term drought treatments on a subset of 12 plots (3m x 3m) in 3  
113 replicate blocks from factorial treatment combinations, namely cessation or continued application of  
114 fertiliser and with or without seed addition. These treatments were selected because the combination of  
115 seed addition and cessation of inorganic fertiliser addition has resulted in the greatest increase in plant  
116 diversity (Smith *et al.* 2008; De Deyn *et al.* 2011).

117 Before the long-term grassland restoration was initiated the meadow received regular additions of  
118 inorganic fertiliser (Smith *et al.* 2000). The fertiliser cessation treatment started in 1990 with the  
119 alternate treatment being continued fertiliser application (NPK 20:10:10; 25kg N ha<sup>-1</sup>) which has since  
120 been applied to plots by hand annually in spring (21 May in 2013), except in 2009 and 2010. Seed  
121 addition treatment also started in 1990 with seed of 19 species coming from locally collected and  
122 commercially bought seed. Since 1999 all plots have been cut for hay between mid-July and August

123 (16 July in 2013), and then grazed by sheep and cattle until hay production in May (Smith *et al.* 2000,  
124 2003, 2008; De Deyn *et al.* 2011). The year prior to establishing the drought experiment, species  
125 richness across 4m<sup>2</sup> was greatest in the fertiliser cessation treatment (26.5±1.4) and lowest with  
126 continued fertiliser application (22.7±1.6; LRT=4.70, d.f.=1,5, P=0.030).

### 127 *Drought treatments*

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

### 144 *Plant community*

145 Aboveground plant biomass was harvested from all plots on 10 July, after the end of the drought  
146 treatment. The plant biomass was dried at 60°C for 48 hours and split by hand into plant functional  
147 types (PFT): grasses, forbs, legumes and hemi-parasitic plants. After ball milling, C and N content for

148 each PFT was measured on a Tru-spec CN analyser (Leco, St. Joseph, MI, USA). Vascular plant species  
149 surveys were carried out between 29 June and 4 July 2013 on the central 706cm<sup>2</sup> of each plot.

#### 150 *CO<sub>2</sub> flux measurements*

151 Net ecosystem exchange (NEE) and ecosystem respiration were measured using static chambers  
152 following Ward *et al.* (2013), linked to an infra-red gas analyser (EGM4, PP Systems, UK), as used by  
153 De Deyn *et al.* (2011). Two minute headspace closures were used for NEE (transparent chambers) and  
154 ecosystem respiration (opaque chambers) between 10:15 and 16:30 hrs alongside measurements of  
155 photosynthetically active radiation (PAR), soil and air temperature. Two measurements were made  
156 before the drought (17, 24 May), six during the experimental drought (13, 20, 24, 27 June; 5, 8 July  
157 2013) and five after shelter removal (19, 26, 30 July; 7, 16 August 2013).

#### 158 *Soil microbial community*

159 Soil was sampled at four time-points with three cores (2.4cm diameter, 10cm depth) taken from each  
160 subplot, bulked together and sieved (2mm). Sampling dates were 5 June (before drought), 10 July  
161 (during drought), 16 August (3 weeks after rewetting) and 4 November (3 months after rewetting).  
162 Before the drought treatment was imposed, samples were only taken from the 12 main plots to determine  
163 treatment effects of seed addition and fertiliser cessation. To quantify belowground biomass, roots were  
164 removed from soil cores sampled during the drought with roots sieved, washed and dried at 60°C for  
165 48 hours before weighing.

166 The effect of grassland restoration and drought on bacterial and fungal community composition was  
167 assessed using the terminal restriction fragment length polymorphism (T-RFLP) method as detailed by  
168 Plassart *et al.* (2012). Genomic DNA was extracted from soil samples using the PowerSoil kit (MoBio,  
169 Carlsbad, US) and amplified using primers for bacterial 16S rRNA and fungal ITS genetic markers. In  
170 addition, broad-scale changes in soil microbial community composition were assessed by phospholipid  
171 fatty acid analysis (PLFA). Briefly, PLFA's were extracted from freeze-dried soil using a modified

172 Bligh-Dyer extraction and separated from other lipids using aminopropyl solid phase extraction  
173 cartridge (Phenomenex, US; White *et al.* 1979). Gas chromatography was carried out on an Agilent  
174 6890GC with fused silica capillary column (Agilent, US). Biomarkers were used for bacteria (i15:0,  
175 a15:0, 15:0, i16:0, 17:0, i17:0, cy-17:0, 18:1 $\omega$ 7 and cy-19:0) and saprotrophic fungi (18:2 $\omega$ 6,9)  
176 (Bardgett, Hobbs & Frostegard 1996; Smith *et al.* 2008).

177 Microbial biomass C and N were measured on 5g fresh soil subsamples using the chloroform  
178 fumigation-incubation method (Brookes *et al.* 1985) in soil cores taken before, during and after  
179 (August) the drought. One subsample was fumigated with chloroform for 16 hours before extraction  
180 with 25mL 0.5M K<sub>2</sub>SO<sub>4</sub>. The resulting filtrate was analysed for microbial C using a TOC analyser  
181 (5000A, Shimadzu, Milton Keynes, UK). For microbial N, filtrate was oxidised with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> before  
182 colorimetric analysis on an autoanalyser (Bran and Luebbe, Northampton, UK). Adjustment factors  
183 were applied, using K<sub>c</sub>=0.35 for microbial C and k<sub>n</sub>=0.54 for microbial N (Bloor & Bardgett 2012). For  
184 soil samples during and after (August) the drought, DOC and DON were extracted from 5g subsamples  
185 using 35mL of water with DOC analysed on a TOC analyser (5000A, Shimadzu, Milton Keynes, UK)  
186 and DON extract oxidised with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> before colorimetric analysis (Bran and Luebbe, Northampton,  
187 UK).

### 188 *Statistical analysis*

189 To investigate responses of plant and soil measurements to drought, we used percentage change caused  
190 by drought and rewetting as indices of resistance and recovery, as widely used in previous studies  
191 (reviewed in Orwin & Wardle 2004). Specifically resistance and recovery were calculated as  $(P_0 - C_0) /$   
192  $C_0 \cdot 100$ , where P<sub>0</sub> is the drought treatment response and C<sub>0</sub> is the control response. Where data were  
193 available, resistance and recovery were calculated using both the ambient and control shelter treatments  
194 as C<sub>0</sub> to allow the effect of the rain-shelter to be investigated.

195 Linear mixed effects models (LME) were used to investigate plant and soil microbial responses to seed  
196 addition, fertiliser and drought treatments. For each LME model the fixed effects were seed, fertiliser,

197 drought and all interactions. The random effect was split-plot nested within block to take account of the  
198 experimental split-plot design. Where data included multiple measurements from a single plot, plot ID  
199 was added as an additional nested random effect. Assumptions of normality and equal variances were  
200 checked graphically and where necessary response variables were either logged or used *varIdent* weight  
201 functions to improve model fit [e.g. *varIdent*(form= $\sim 1$ |fertiliser)], following Zuur *et al.* (2009). We  
202 determined the significance of fixed effects by comparing models with and without the factor of interest  
203 using likelihood ratio tests (LRT). To investigate changes in soil microbial community structure, the  
204 relative abundance of T-RF peaks was assessed using between-sample Bray-Curtis dissimilarities and  
205 non-metric multidimensional scaling with permutational multivariate analysis of variance. All statistical  
206 analysis was carried out in the R programming language 3.3.1 (R Core Development Team, 2016) using  
207 the *nlme* package for mixed effect models (Pinheiro *et al.* 2013).

## 208 **Results**

### 209 *Rain shelter effects on soil moisture and temperature*

210 The drought treatment excluded 180.8mm of rainfall over 35 days with significant rewetting occurring  
211 in late July, more than a week after rain-out shelters were removed (Fig 1b). The treatment intercepted  
212 rainfall equivalent to 7.5% of average annual precipitation and reduced soil moisture from 58.8% to  
213 33.3%, while the roofed control shelter also intercepted some rainfall and reduced soil moisture from  
214 58.8% to 49.8% (Fig 1a, LRT=113.55, d.f.=2,10,  $P<0.0001$ ). Additionally, fertiliser application reduced  
215 soil moisture by 3% relative to cessation of fertiliser (LRT=5.68, d.f.=1,11,  $P=0.017$ ). Two nearby sites  
216 with long-term precipitation datasets suggest that 100-year extreme drought events in the primary  
217 growing season equate to 27 and 34 days with <1mm rainfall (Bloor & Bardgett 2012), similar to the  
218 35 days rain-out shelters were in place in this study. No effects of rain-out shelters were detected on  
219 mean air temperature (LRT=2.45, d.f.=2,6,  $P=0.293$ ) or soil temperature (LRT=1.72, d.f.=2,7,  
220  $P=0.424$ ).

221 *Plant community*

222 Cessation of fertiliser use brought about an average 1.2 species increase in plant species richness  
223 (LRT=6.46, d.f.=1,10,  $P=0.011$ ), while seed addition increased species richness, but only in the ambient  
224 treatment which had no rain shelter (Fig 2a, drought x seed: LRT=8.59, d.f.=2,13,  $P=0.014$ ). Total  
225 aboveground biomass, harvested in July, was 49.4% lower in non-fertilised compared to fertilised plots  
226 (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,  
227  $P=0.244$ ). For specific plant functional types, grass and forb biomass was respectively 57.2% and 35.2%  
228 lower in non-fertilised relative to fertilised plots (Fig 3, Grass: LRT=19.02, d.f.=1,10,  $P<0.0001$ ; Forb:  
229 LRT=9.43, d.f.=1,11,  $P=0.002$ ), while hemi-parasitic plant biomass was nearly double (Fig 3d,  
230 LRT=3.94, d.f.=1,10,  $P=0.047$ ). As a consequence, the proportion of plant functional groups shifted in  
231 non-fertilised plots, with proportionally less grass biomass and more legume and hemi-parasitic plant  
232 biomass (Fig S1).

233 Drought, superimposed across the long-term grassland restoration treatments, did not reduce total  
234 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,  
235  $P=0.231$ ). However, legume biomass increased under drought (Fig 2c, LRT=8.00, d.f.=2,11,  $P=0.018$ ),  
236 whereas for grasses drought had no impact, although forb biomass was marginally increased by drought  
237 but only in plots with seed addition (Fig 3). Additionally, the hemi-parasitic plant species *R. minor* was  
238 more abundant in plots without than with seed addition, but primarily so under drought conditions (Fig  
239 3e, drought x seed: LRT=6.43, d.f.=2,13,  $P=0.040$ ). Furthermore, in grasslands with seed addition the  
240 resistance of N content in shoot biomass was increased across all forbs, grasses and legumes (Fig 4a,  
241 LRT=5.52, d.f.=1,12,  $P=0.019$ ).

242 *CO<sub>2</sub> fluxes*

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

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

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

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

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

### 277 *Soil properties and microbial community*

278 Microbial biomass C and N were partially reduced by drought (Fig S5), with reduced resistance in  
279 grasslands with seed addition (Fig 4b, microbial biomass C: LRT=12.47, d.f.=1,5,  $P=0.0004$ ; microbial  
280 biomass N: LRT=4.79, d.f.=1,6,  $P=0.029$ ). Three weeks after the drought, both microbial biomass C  
281 and N showed full recovery to levels in non-droughted plots (Fig S5, 4c). In contrast to soil DOC, soil  
282 DON was partially reduced by drought (Fig S6), with reduced resistance in grasslands without seed  
283 addition (Fig 4b, LRT=4.71, d.f.=1,5,  $P=0.030$ ). However after rewetting, recovery of DON was almost  
284 4 fold greater with continued fertiliser application (Fig 4c, LRT=6.07, d.f.=1,7,  $P=0.014$ ).

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

## 292 **Discussion**

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

301 In this study, drought was superimposed on a 23-year grassland restoration experiment in which seed  
302 addition and fertiliser cessation have brought about the greatest increase in plant diversity (Smith *et al.*  
303 2003, 2008). Following previous studies, fertiliser cessation and seed addition have continued to bring  
304 about generally small increases in species richness, alongside shifts in plant functional groups from  
305 grasses to legumes and hemi-parasitic plants and an increase in soil fungi relative to bacteria (Smith *et*  
306 *al.* 2000, 2003, 2008).

307 The impact of drought on grasslands subject to biodiversity restoration management was tested using  
308 both a complete rain shelter and a control shelter with holes to test for any unanticipated effects of the  
309 shelter (Vogel *et al.* 2013). The control shelter reduced soil moisture by 9% making it difficult to  
310 separate unanticipated side-effects of the shelter from those caused by the reduced soil moisture. Even  
311 so, any unanticipated artefacts of the shelter were most likely to be seen in CO<sub>2</sub> fluxes, which were  
312 sensitive to PAR and soil temperature, and which can be impacted by shelters (Vogel *et al.* 2013). In  
313 contrast, the reduction in ecosystem respiration under drought was similar irrespective of whether it  
314 was relative to the ambient or control shelter treatment (Fig 6). This suggests that results are unlikely  
315 to be impacted by unanticipated artefacts of the rain shelter, with overall conclusions robust to changing  
316 how drought data are included in statistical models (Table S2, S3). Therefore the subset of

317 measurements that were not able to include the extra level of control are still likely to represent the  
318 effect of drought rather than the shelter.

### 319 *Drought and plant community*

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

### 336 *Drought and CO<sub>2</sub> fluxes*

337 As hypothesised (H1), drought reduced ecosystem respiration by 12.3% in grasslands with fertiliser  
338 addition but with minimal impact in grasslands with cessation of fertiliser. Additionally, this result was  
339 robust to the use of either ambient or control shelter treatments to calculate resistance indices. The  
340 greater resistance of ecosystem respiration to drought was likely due to low productivity grasslands  
341 being more resistant to drought, due to reduced water demand (Wang, Yu & Wang 2007). This suggests

342 a potential trade-off between agricultural yields and resistance to drought. In this long-term experiment,  
343 hay yields reduced as *R. minor* became abundant 6 years after initial seed addition (Smith *et al.* 2000),  
344 suggesting hemi-parasitic plants may increase resistance of C cycling to drought through reducing plant  
345 productivity, in addition to enabling slow-growing species to establish (Joshi *et al.* 2000, Bardgett *et al.*  
346 2006). These conclusions, however, will differ where grassland biodiversity restoration has been found  
347 to increase agricultural yields, for example in seeding experiments on ex-arable land (Bullock *et al.*  
348 2001). This suggests there may be variability in the impact of grassland biodiversity restoration on  
349 community resistance to drought, primarily due to the impact of restoration on agricultural yield.

### 350 *Drought and soil microbial community*

351 Drought reduced microbial biomass C and N in plots with seed addition, suggesting the drought  
352 response was modulated by the plant community composition (Bloor & Bardgett 2012; De Vries *et al.*  
353 2018). Additionally, the increase in fungal PLFAs under drought may point to a strong link between  
354 plants and fungi under drought (Fuchslueger *et al.* 2014), potentially due to plants accessing water  
355 through the arbuscular mycorrhiza hyphal networks (Karlowsky *et al.* 2017). Unanticipated artefacts of  
356 the rain shelter, however, cannot be ruled out, due to not having data on soil microbial communities  
357 from the control shelter treatment. Nevertheless, our study suggests that the increase in the abundance  
358 of soil fungi over two decades of biodiversity restoration has led to a soil microbial community that is  
359 resistant to drought.

360 The soil microbial community showed high resilience after rewetting, irrespective of grassland  
361 biodiversity restoration treatment. Three weeks after rewetting, microbial biomass C and N had fully  
362 recovered, while the effect of drought on microbial community structure persisted. Additionally a large  
363 post-rewetting increase in soil DON followed similar increases in soil N availability post drought (Roy  
364 *et al.* 2016). Recovery of the soil microbial community after drought can be variable, with different  
365 microbial groups responding differently (De Vries *et al.* 2012b; Fuchslueger *et al.* 2014; De Vries *et al.*  
366 2018); however our data show that microbial biomass C and N can recover more quickly than microbial  
367 community structure and this high resilience is irrespective of grassland restoration treatment.

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

380 Research shows that grassland restoration can increase soil C accumulation (De Deyn *et al.* 2011),  
381 nutrient retention (Maron & Jefferies 2001), and resistance of plant productivity and C fluxes to drought  
382 (Wang *et al.* 2007; this study). Yet these benefits, and those found here, were all found to be associated  
383 with lower productivity grasslands or cessation of fertiliser application. This suggests multiple trade-  
384 offs between broader multifunctionality and agricultural yield when restoring grassland botanical  
385 diversity. Grassland restoration is commonly supported by agri-environment schemes, however  
386 payments are often linked to set estimates of income forgone rather than for the public goods they  
387 deliver (Fraser 2009). In the context of grassland restoration, the actual income forgone will be greater  
388 where restoration leads to low yields which may make payments less attractive to the land owner, but  
389 confer additional benefits (e.g. Wang *et al.* 2007). In contrast, grassland restoration which increases  
390 agricultural yields will have minimal actual income forgone (Bullock *et al.* 2007), and may appear more  
391 attractive. This suggests a need to disentangle the trade-offs between the benefits of grassland  
392 restoration to the public (e.g. C sequestration) and land manager (e.g. productivity resistant to drought),  
393 and to what extent this may change under a future climate.

## 394 **Conclusions**

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

## 408 **Authors' contributions**

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

## 412 **Acknowledgements**

413 Long-term precipitation data was provided by Malham Tarn field study centre. AJC was funded by  
414 NERC studentship (NE/K500951/1), supervised by RIG, JW, NO and RDB. We thank Bruce Thomson  
415 for assistance with T-RFLP analysis and Natural England staff at Colt Park for their assistance in  
416 running the experiment. The field experiment used in this study was supported by funds from DEFRA

417 (project number BD5003) awarded to RDB. We thank two anonymous reviewers and Associate Editor  
418 who helped improve the paper.

#### 419 **Data accessibility**

420 Data available via the Environmental Information Data Centre [https://doi.org/10.5285/8a41b2a2-01d7-](https://doi.org/10.5285/8a41b2a2-01d7-409e-adf5-fba3f3770f29)  
421 [409e-adf5-fba3f3770f29](https://doi.org/10.5285/8a41b2a2-01d7-409e-adf5-fba3f3770f29) (Cole *et al.* 2019).

#### 422 **References**

- 423 Bardgett, R.D., Hobbs, P.J. & Frostegard, A. (1996) Changes in soil fungal:bacterial biomass ratios  
424 following reductions in the intensity of management of an upland grassland. *Biology and Fertility*  
425 *of Soils*, **22**, 261–264.
- 426 Bardgett, R.D. & McAlister, E. (1999) The measurement of soil fungal:bacterial biomass ratios as an  
427 indicator of ecosystem self-regulation in temperate meadow grasslands. *Biology and Fertility of*  
428 *Soils*, **29**, 282–290.
- 429 Bardgett, R.D., Smith, R.S., Shiel, R.S., Peacock, S., Simkin, J.M., Quirk, H. & Hobbs, P.J. (2006)  
430 Parasitic plants indirectly regulate below-ground properties in grassland ecosystems. *Nature*, **439**,  
431 969–72.
- 432 Bloor, J.M.G. & Bardgett, R.D. (2012) Stability of above-ground and below-ground processes to  
433 extreme drought in model grassland ecosystems: Interactions with plant species diversity and soil  
434 nitrogen availability. *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 193–204.
- 435 Brookes, P.C., Landman, A., Pruden, G. & Jenkinson, D.S. (1985) Chloroform fumigation and the  
436 release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen  
437 in soil. *Soil Biology and Biochemistry*, **17**, 837–842.
- 438 Bullock, J.M., Pywell, R.F., Burke, M.J.W. & Walker, K.J. (2001) Restoration of biodiversity enhances  
439 agricultural production. *Ecology Letters*, **4**, 185–189.
- 440 Carter, D.L. & Blair, J.M. (2012) High richness and dense seeding enhance grassland restoration  
441 establishment but have little effect on drought response. *Ecological Applications*, **22**, 1308–1319.
- 442 Cole, A.J., Griffiths, R.I., Ward, S.E., Whitaker, J., Ostle, N.J., Bardgett, R.D. (2019). Plant and soil  
443 responses to simulated summer drought in 2013 on Colt Park grassland restoration experiment.  
444 NERC Environmental Information Data Centre. [https://doi.org/10.5285/8a41b2a2-01d7-409e-](https://doi.org/10.5285/8a41b2a2-01d7-409e-adf5-fba3f3770f29)  
445 [adf5-fba3f3770f29](https://doi.org/10.5285/8a41b2a2-01d7-409e-adf5-fba3f3770f29)
- 446 De Deyn, G.B., Shiel, R.S., Ostle, N.J., McNamara, N.P., Oakley, S., Young, I., ... Bardgett, R.D.  
447 (2011) Additional carbon sequestration benefits of grassland diversity restoration. *Journal of*  
448 *Applied Ecology*, **48**, 600–608.
- 449 De Vries, F.T., Bloem, J., Quirk, H., Stevens, C.J., Bol, R. & Bardgett, R.D. (2012a) Extensive  
450 Management Promotes Plant and Microbial Nitrogen Retention in Temperate Grassland. *PLoS*  
451 *ONE*, **7**, e51201.
- 452 De Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M. & Bardgett,  
453 R.D. (2012b) Land use alters the resistance and resilience of soil food webs to drought. *Nature*  
454 *Climate Change*, **2**, 276–280.
- 455 De Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., ... & Lemanceau, P.

- 456 (2018) Soil bacterial networks are less stable under drought than fungal networks. *Nature*  
457 *Communications*, **9**, 3033.
- 458 Donohue, I., Hillebrand, H., Montoya, J.M., Petchey, O.L., Pimm, S.L., Fowler, M.S., ... Yang, Q.  
459 (2016) Navigating the complexity of ecological stability. *Ecology letters*, **19**, 1172–1185.
- 460 Fraser, R. (2009) Land heterogeneity, agricultural income forgone and environmental benefit: An  
461 assessment of incentive compatibility problems in environmental stewardship schemes. *Journal*  
462 *of agricultural economics*, **60**, 190-201.
- 463 Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R. & Richter, A. (2014) Experimental drought reduces  
464 the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community  
465 composition in a mountain meadow. *New Phytologist*, **201**, 916–927.
- 466 Grieu, P., Lucero, D.W., Ardiani, R. & Ehleringer, J.R. (2001) The mean depth of soil water uptake by  
467 two temperate grassland species over time subjected to mild soil water deficit and competitive  
468 association. *Plant and Soil*, **230**, 197–209.
- 469 Harper, C.W., Blair, J.M., Fay, P.A., Knapp, A.K. & Carlisle, J.D. (2005) Increased rainfall variability  
470 and reduced rainfall amount decreases soil CO<sub>2</sub> flux in a grassland ecosystem. *Global Change*  
471 *Biology*, **11**, 322–334.
- 472 Hoekstra, N.J., Finn, J.A., Hofer, D. & Lüscher, A. (2014) The effect of drought and interspecific  
473 interactions on depth of water uptake in deep- and shallow-rooting grassland species as determined  
474 by  $\delta^{18}\text{O}$  natural abundance. *Biogeosciences*, **11**, 4493–4506.
- 475 Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N. & Lüscher, A. (2016) Yield  
476 of temperate forage grassland species is either largely resistant or resilient to experimental summer  
477 drought. *Journal of Applied Ecology*, 1023–1034.
- 478 IPCC (2013) Climate change 2013: The physical science basis. In Stocker, T.F., Qin, D., Plattner, G.K.,  
479 Tignor, M., Allen, S.K., Boschung, J., ... Midgley, P.M. (Eds.), *Contribution of working group I*  
480 *to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge, UK:  
481 Cambridge University Press.
- 482 Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., ... Eisenhauer, N. (2015)  
483 Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature*, **526**,  
484 574–577.
- 485 Joshi, J., Matthies, D., & Schmid, B. (2000) Root hemiparasites and plant diversity in experimental  
486 grassland communities. *Journal of Ecology*, **88**, 634-644.
- 487 Karlowsky, S., Augusti, A., Ingrisch, J., Hasibeder, R., Lange, M., Lavorel, S., ... Gleixner, G. (2018)  
488 Land use in mountain grasslands alters drought response and recovery of carbon allocation and  
489 plant-microbial interactions. *Journal of Ecology*, 1–14.
- 490 Maron, J.L. & Jefferies, R.L. (2001) Restoring enriched grasslands: effects of mowing on species  
491 richness, productivity and nitrogen retention. *Ecological Applications*, **11**, 1088–1100.
- 492 Morecroft, M. D., Masters, G. J., Brown, V. K., Clarke, I. P., Taylor, M. E., & Whitehouse, A. T. (2004)  
493 Changing precipitation patterns alter plant community dynamics and succession in an ex-arable  
494 grassland. *Functional ecology*, **18**, 648-655.
- 495 Oliver, T.H., Heard, M.S., Isaac, N.J.B., Roy, D.B., Procter, D., Eigenbrod, F., ... Bullock, J.M. (2015)  
496 Biodiversity and Resilience of Ecosystem Functions. *Trends in Ecology & Evolution*, **xx**, 1–12.
- 497 Orwin, K.H. & Wardle, D.A. (2004) New indices for quantifying the resistance and resilience of soil  
498 biota to exogenous disturbances. *Soil Biology and Biochemistry*, **36**, 1907–1912.
- 499 Plassart, P., Terrat, S., Thomson, B.C., Griffiths, R.I., Dequiedt, S., Lelievre, M., ... Ranjard, L. (2012)  
500 Evaluation of the ISO standard 11063 DNA extraction procedure for assessing soil microbial  
501 abundance and community structure. *PloS one*, **7**, e44279.
- 502 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2013) *nlme: Linear and Nonlinear*

503 *Mixed Effects Models*. R package version 3.1-113

504 Roy, J., Picon-Cochard, C., Augusti, A., Benot, M.-L., Thiery, L., Darsonville, O., ... Soussana, J.-F.  
505 (2016) Elevated CO<sub>2</sub> maintains grassland net carbon uptake under a future heat and drought  
506 extreme. *Proceedings of the National Academy of Sciences*, **113**, 6224–6229.

507 Schimel, J.P., Balsler, T.C. & Wallenstein, M.D. (2007) Microbial Stress-Response Physiology and Its  
508 Implications for Ecosystem. *Ecology*, **88**, 1386–1394.

509 Smith, R.S., Shiel, R.S., Bardgett, R.D., Millward, D., Corkhill, P., Evans, P., ... Kometa, S.T. (2008)  
510 Long-term change in vegetation and soil microbial communities during the phased restoration of  
511 traditional meadow grassland. *Journal of Applied Ecology*, **45**, 670–679.

512 Smith, R.S., Shiel, R.S., Bardgett, R.D., Millward, D., Corkhill, P., Rolph, G., ... Peacock, S. (2003)  
513 Soil microbial community, fertility, vegetation and diversity as targets in the restoration  
514 management of a meadow grassland. *Journal of Applied Ecology*, **40**, 51–64.

515 Smith, R.S., Shiel, R.S., Millward, D. & Corkhill, P. (2000) The interactive effects of management on  
516 the productivity and plant community structure of an upland meadow: an 8-year field trial.  
517 *Journal of Applied Ecology*, **37**, 1029–1043.

518 Vogel, A., Fester, T., Eisenhauer, N., Scherer-Lorenzen, M., Schmid, B., Weisser, W.W. & Weigelt, A.  
519 (2013) Separating drought effects from roof artifacts on ecosystem processes in a grassland  
520 drought experiment. *PloS one*, **8**, e70997.

521 Wang, Y., Yu, S. & Wang, J. (2007) Biomass-dependent susceptibility to drought in experimental  
522 grassland communities. *Ecology Letters*, **10**, 401–410.

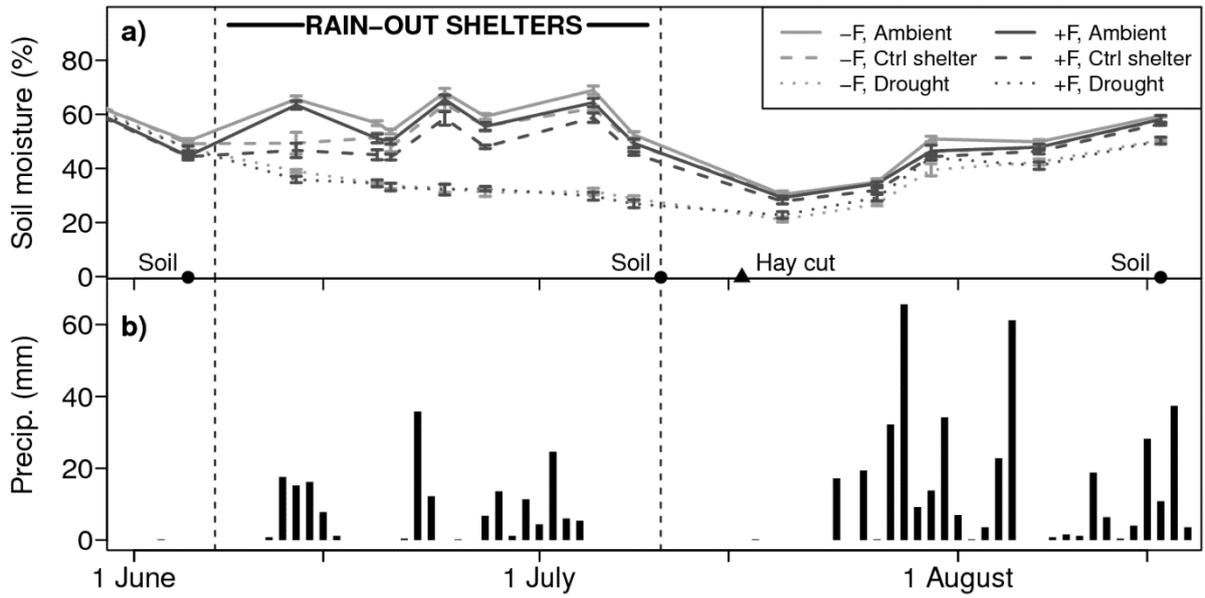
523 Ward, S.E., Ostle, N.J., Oakley, S., Quirk, H., Henrys, P.A. & Bardgett, R.D. (2013) Warming effects  
524 on greenhouse gas fluxes in peatlands are modulated by vegetation composition. *Ecology Letters*,  
525 **16**, 1285–1293.

526 White, D.C., Davies, J.S., Nickels, J.S., King, J.D. & Bobbie, R.J. (1979) Determination of the  
527 sedimentary microbial biomass by extractible lipid phosphate. *Oecologia*, **40**, 51–62.

528 Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., & Smith, G.M. (2009) *Mixed effects models and*  
529 *extensions in ecology with R*. New York, NY: Springer.

530

531



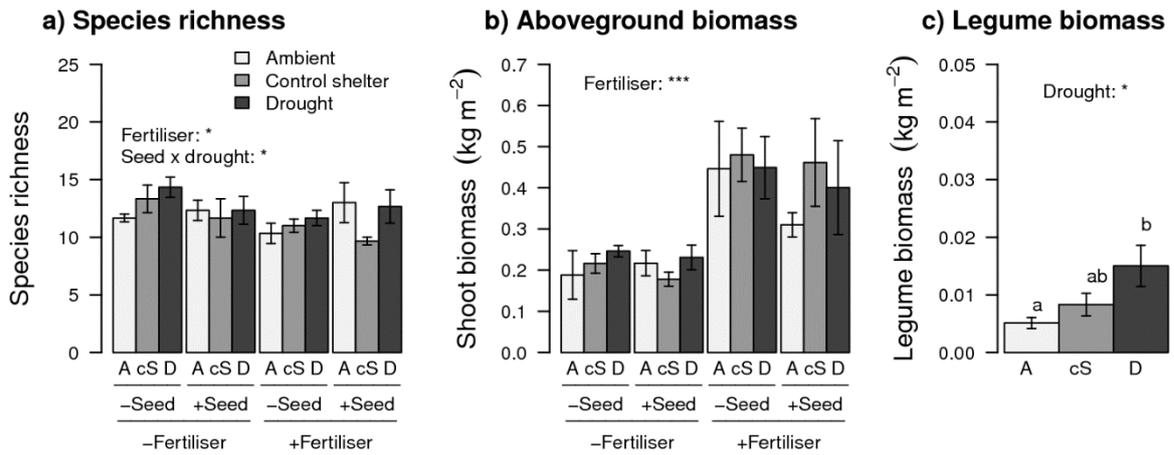
532

533

534 **Figure 1.** Soil moisture (a) and daily precipitation (b) through the experiment. -F represents fertiliser

535 cessation, +F represents continued fertiliser application. Central x-axis indicates soil sampling (circles)

536 and hay cut (triangle). Vertical dashed lines show installation and removal of rain-out shelters.



537

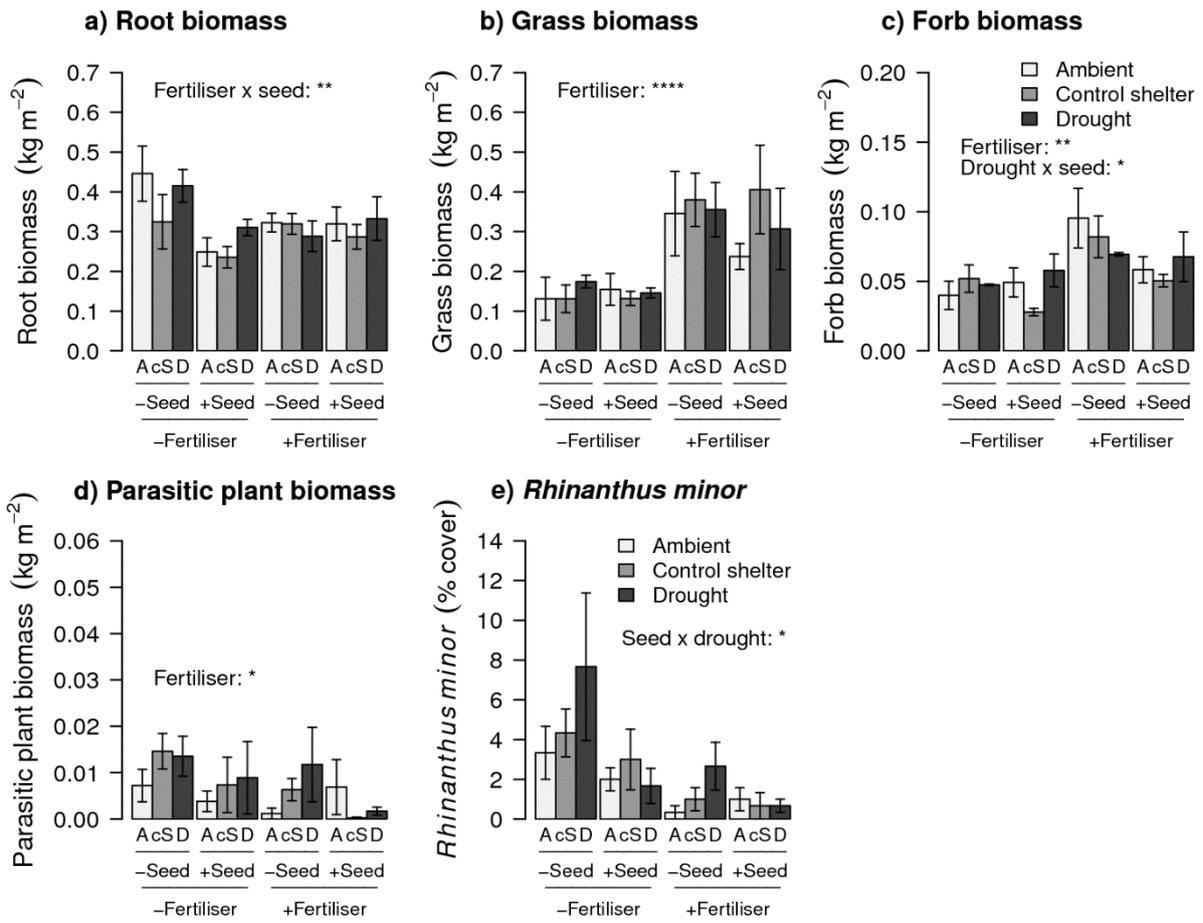
538 **Figure 2.** Plant community responses to biodiversity restoration treatments and drought; (a) species

539 richness; (b) shoot biomass; (c) legume biomass, for ambient (A), control shelter (cS) and drought (D)

540 treatments. The significance of main effects and interactions indicated by \*\*\*= $P < 0.001$ , \*\*= $P < 0.01$ ,

541 \*= $P < 0.05$  and for panel (c) different letters represent significant differences between treatments.

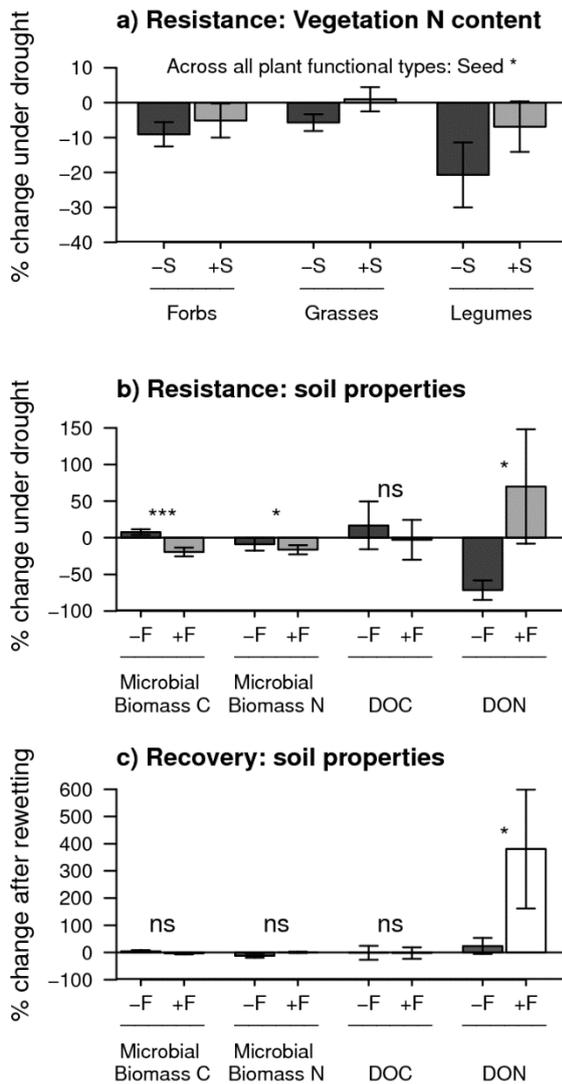
542



544

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

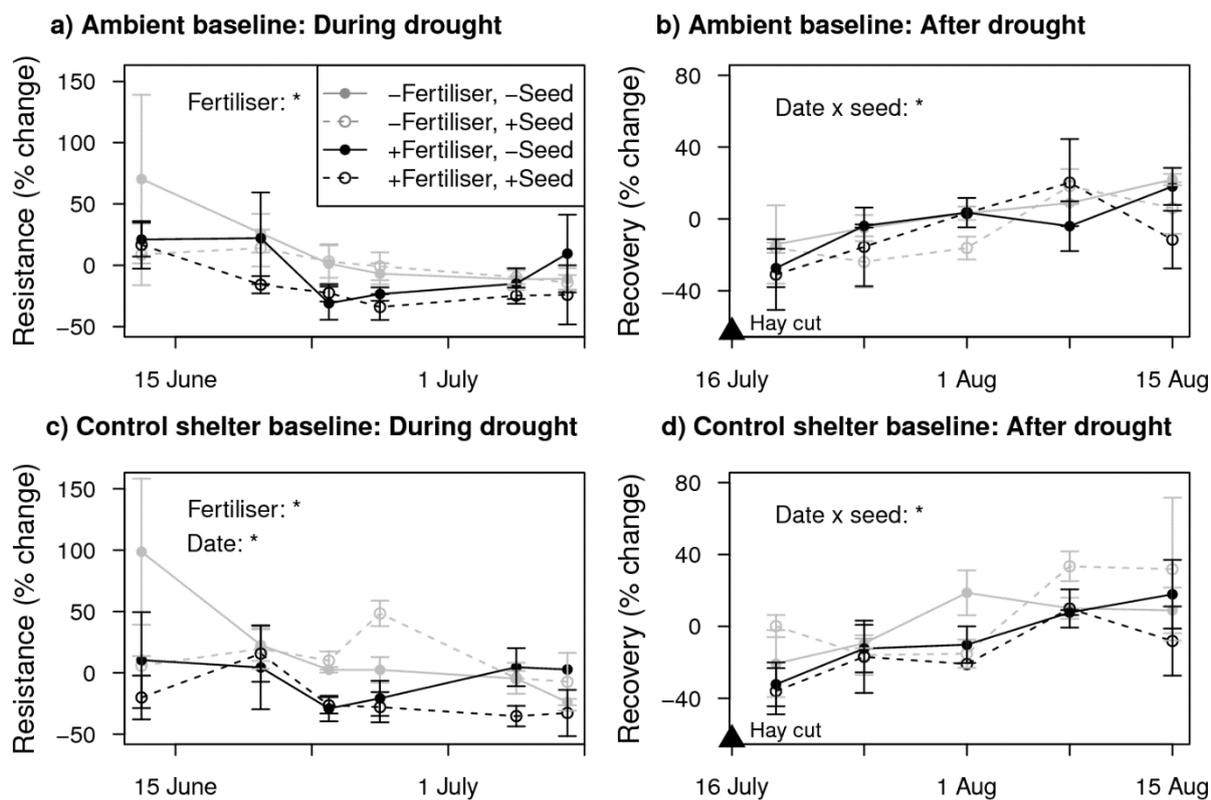
549



550

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

556  
 557



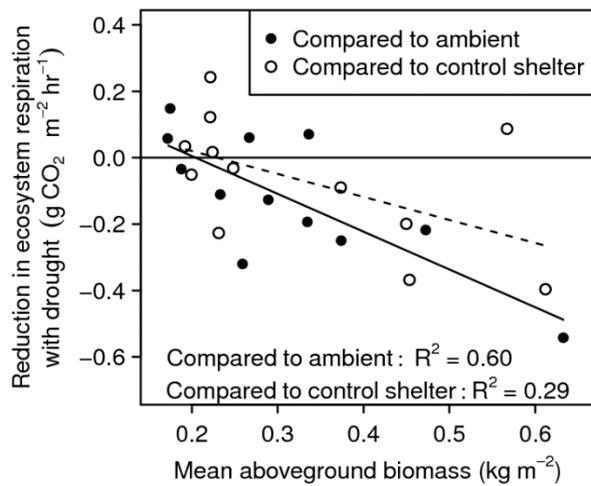
559

560 **Figure 5.** The resistance and recovery of ecosystem respiration to drought using the ambient treatment  
 561 as reference treatment (a-b) and the control shelter treatment as reference treatment (c-d). The  
 562 significance of main effects and interactions indicated by \*=P<0.05.

563

564

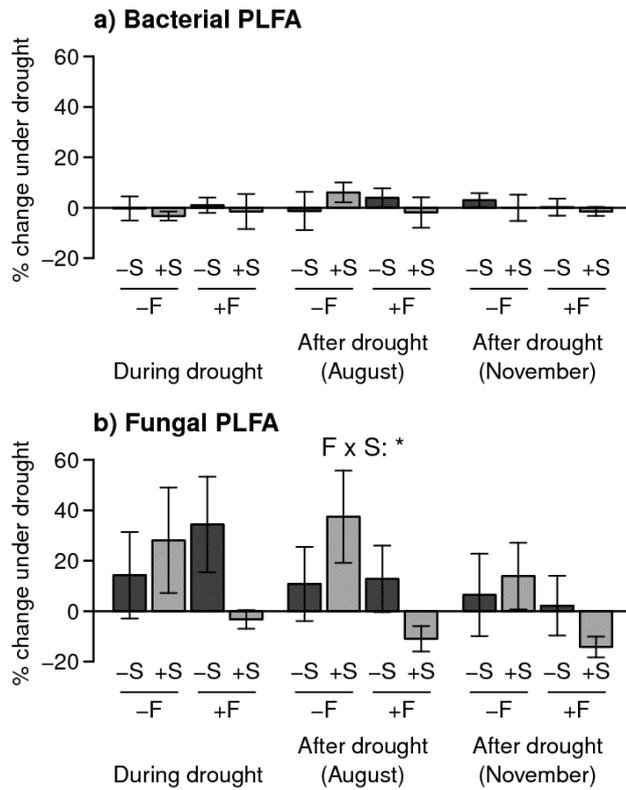
565



566

567 **Figure 6.** Reduction in ecosystem respiration with drought averaged across sampling from 20 June to  
568 5 July. Dashed horizontal line indicates the same ecosystem respiration in ambient and drought.

569



570

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

576