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

2 **drought**

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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.

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 CO_2 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.

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 of 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*

90   *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^{-1}) 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 $4m^2$ 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).

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.

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. Joesph, MI, USA). Vascular plant species

149   surveys were carried out between 29 June and 4 July 2013 on the central 706cm^2 of each plot.

CO$_2$ 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).

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 16SrRNA 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 (Phenonenex, 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, 18:1ω7 and cy-19:0) and saprotrophic fungi (18:2ω6,9)

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_2SO_4. 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 $K_2S_2O_8$ before

182 colorimetric analysis on an autoanalyser (Bran and Luebbe, Northampton, UK). Adjustment factors

183 were applied, using K_c=0.35 for microbial C and k_n=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 $K_2S_2O_8$ 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_0.100, where P_0 is the drought treatment response and C_0 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_0 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=~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).

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).

CO$_2$ 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,

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 win

291   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 CO_2 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

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$_2$ 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 suuported 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.

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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419 **Data accessibility**

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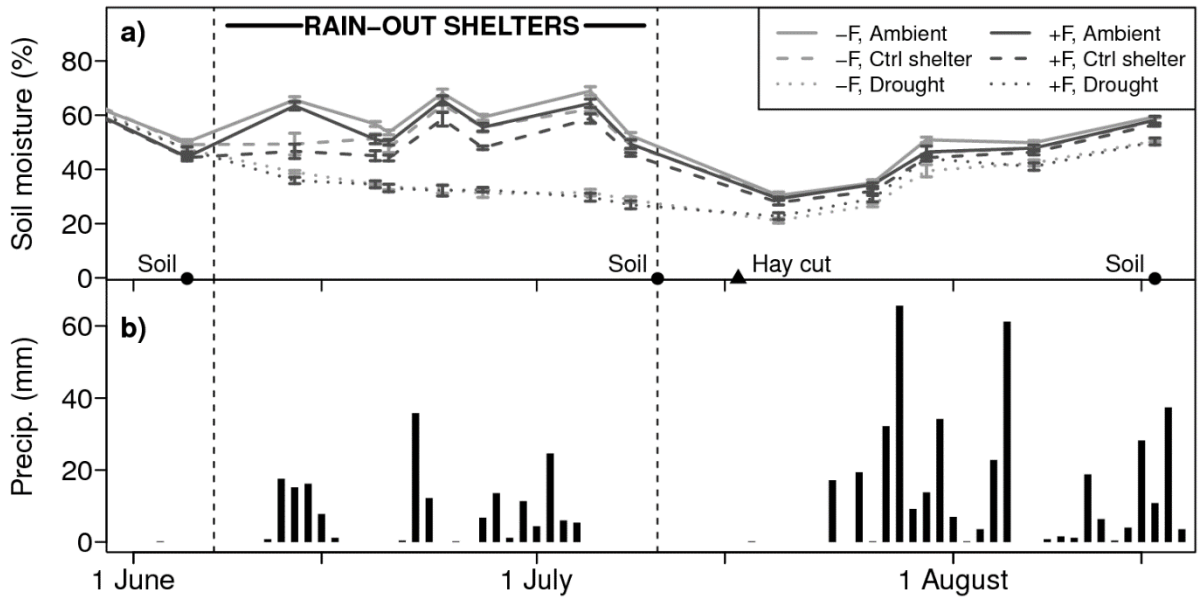
Figure 1. Soil moisture (a) and daily precipitation (b) through the experiment. –F represents fertiliser cessation, +F represents continued fertiliser application. Central x-axis indicates soil sampling (circles) and hay cut (triangle). Vertical dashed lines show installation and removal of rain-out shelters.
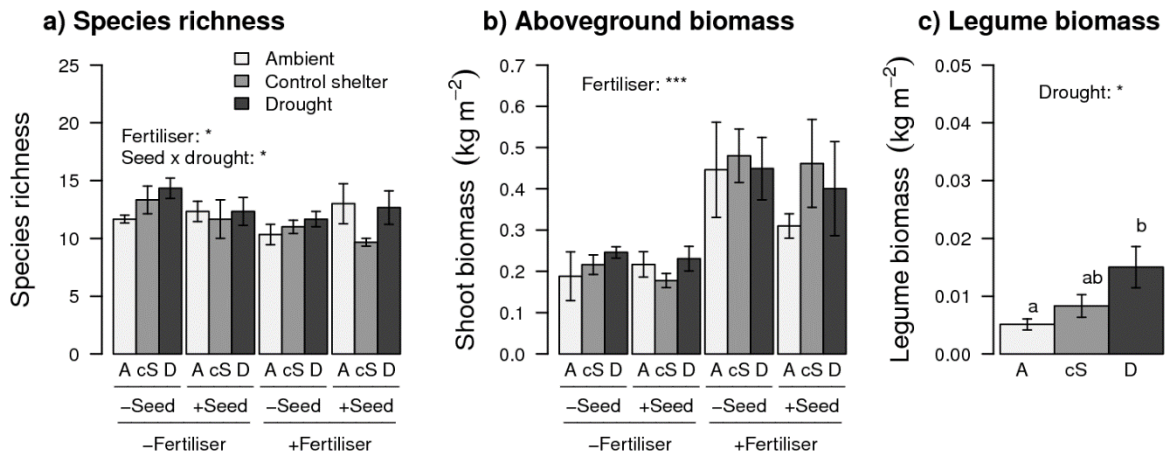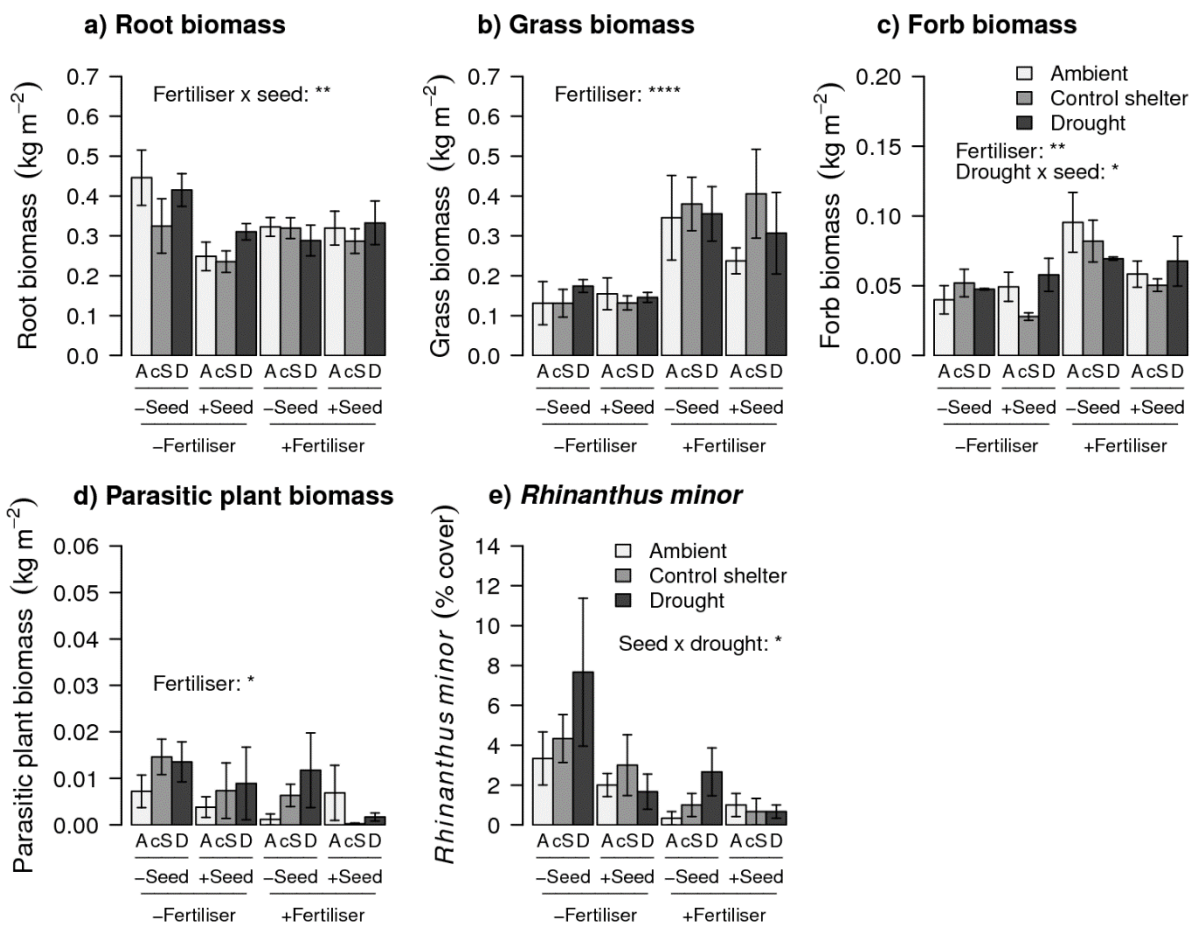
Figure 2. Plant community responses to biodiversity restoration treatments and drought; (a) species richness; (b) shoot biomass; (c) legume biomass, for ambient (A), control shelter (cS) and drought (D) treatments. The significance of main effects and interactions indicated by ***=P<0.001, **=P<0.01, *=P<0.05 and for panel (c) different letters represent significant differences between treatments.
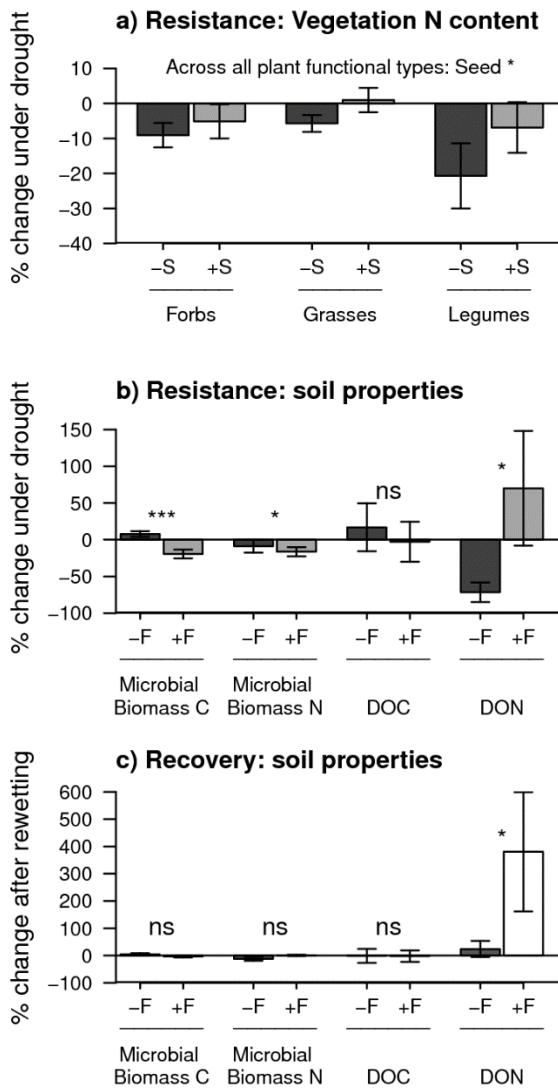
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545 **Figure 3.** Plant community responses to restoration treatments and drought; (a) root biomass; (b) grass

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.

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a) Resistance: Vegetation N content

b) Resistance: soil properties

c) Recovery: soil properties
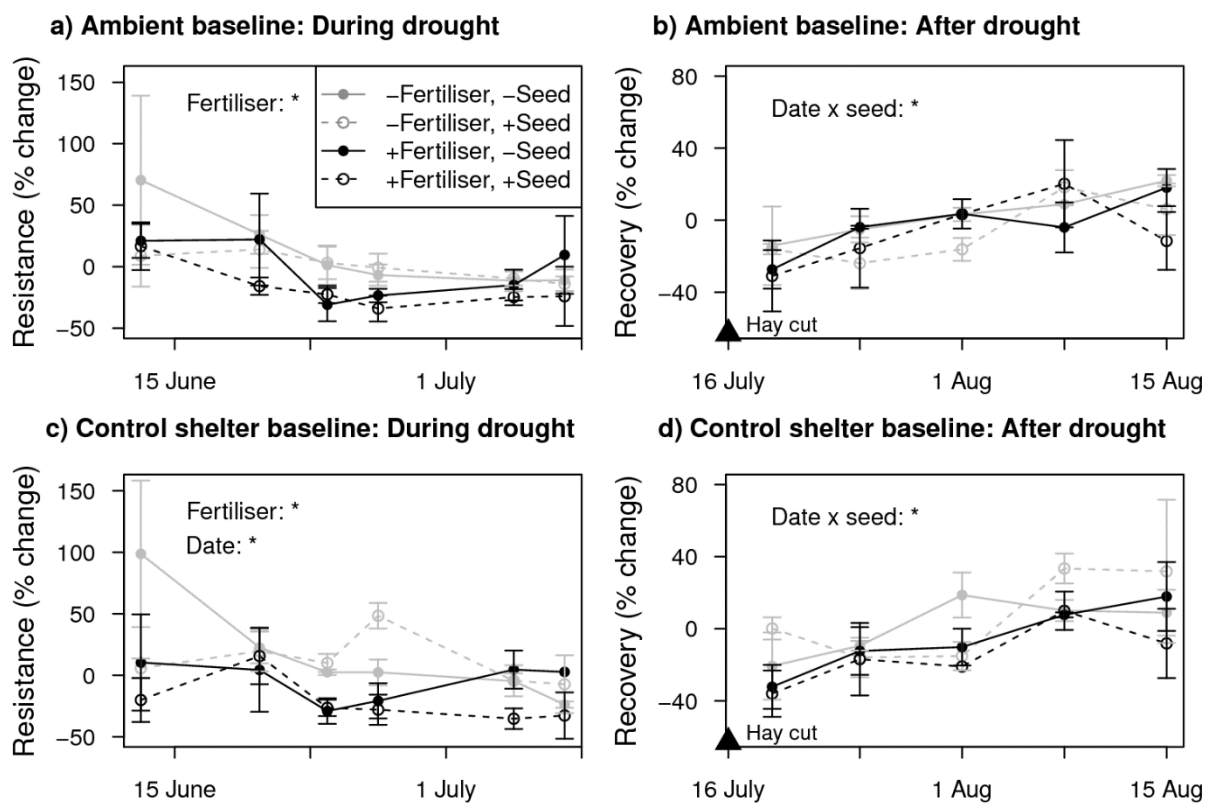
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Figure 4. Resistance (a) vegetation N content to drought and resistance (b) and recovery (c) of soil microbial and organic C and N pools to drought. Assessed as percentage change where positive values show increases under drought or following re-wetting. –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

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a) Ambient baseline: During drought

b) Ambient baseline: After drought

c) Control shelter baseline: During drought
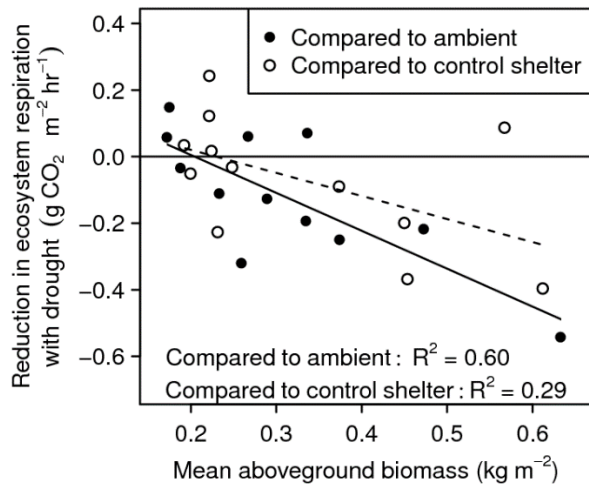
d) Control shelter baseline: After drought

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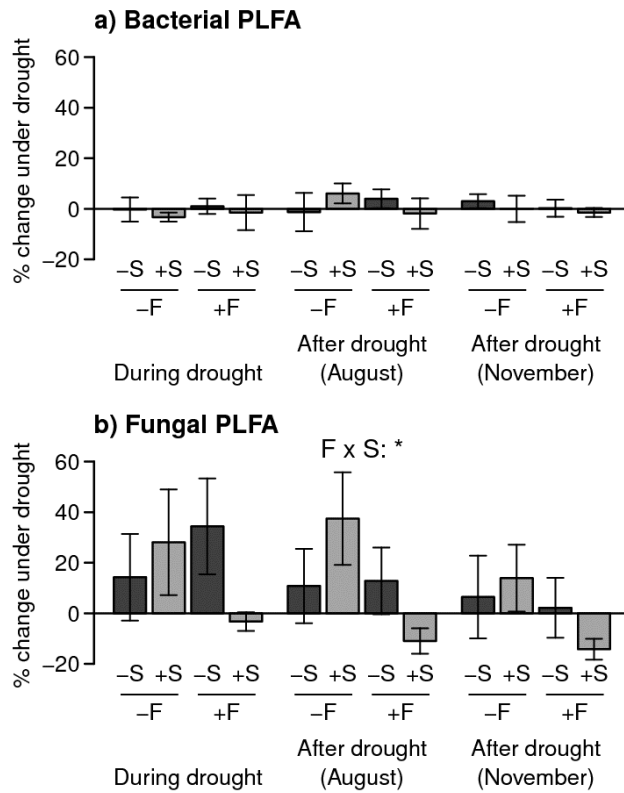
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Figure 6. Reduction in ecosystem respiration with drought averaged across sampling from 20 June to
5 July. Dashed horizontal line indicates the same ecosystem respiration in ambient and drought.

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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.