

#### Resilience of ecosystem service delivery in grasslands in response to single and compound extreme weather events

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## 1 Highlights

- 2 Flood and drought events had negative impact on indicators of ecosystem function
  - This grassland was more resistant and resilient to drought than flood
  - Flooding led to pronounced and persistent shift in plant and microbial communities
  - The combination of flood and drought stress increased the resilience of the system
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7	Resilience of ecosystem service delivery in grasslands in response to single and
8	compound extreme weather events

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#### 32 Abstract

Extreme weather events are increasing in frequency and magnitude with profound effects on 33 34 ecosystem functioning. Further, there is now a greater likelihood that multiple extreme events are occurring within a single year. Here we investigated the effect of a single drought, flood 35 or compound (flood+drought) extreme event on temperate grassland ecosystem processes in a 36 field experiment. To assess system resistance and resilience, we studied changes in a wide 37 38 range of above- and below-ground indicators (plant diversity and productivity, greenhouse gas emissions, soil chemical, physical and biological metrics) during the 8 week stress events 39 40 and then for 2 years post-stress. We hypothesized that agricultural grasslands would have different degrees of resistance and resilience to flood and drought stress. We also investigated 41 two alternative hypotheses that the combined flood+drought treatment would either, (A) 42 promote ecosystem resilience through more rapid recovery of soil moisture conditions or (B) 43 exacerbate the impact of the single flood or drought event. Our results showed that flooding 44 had a much greater effect than drought on ecosystem processes and that the grassland was 45 more resistant and resilient to drought than to flood. The immediate impact of flooding on all 46 indicators was negative, especially for those related to production, and climate and water 47 regulation. Flooding stress caused pronounced and persistent shifts in soil microbial and plant 48 communities with large implications for nutrient cycling and long-term ecosystem function. 49 The compound flood+drought treatment failed to show a more severe impact than the single 50 51 extreme events. Rather, there was an indication of quicker recovery of soil and microbial parameters suggesting greater resilience in line with hypothesis (A). This study clearly 52 reveals that contrasting extreme weather events differentially affect grassland ecosystem 53 function but that concurrent events of a contrasting nature may promote ecosystem resilience 54 55 to future stress.

56 Keywords: Climate change, soil quality, drought, flooding, greenhouse gas emissions,
57 microbial community

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#### 59 **1. Introduction**

Grasslands represent an important global agroecosystem covering an area of ca. 11 million 60 km<sup>2</sup> and 7 % of the earth's terrestrial area (O'Mara, 2012). In addition to food production, 61 62 grasslands provide a wide range of ecosystem services (ES), such as biodiversity provision, climate regulation and natural hazard protection (MEA, 2005; O'Mara, 2012) which rely on 63 64 effective belowground soil functioning (Adhikari and Hartemink, 2016; Brussaard, 1997; Wagg et al., 2014). The effects of climate change are already being felt, with the frequency 65 and severity of extreme weather events increasing in every region of the globe (IPCC, 2021). 66 67 Such extreme events have been shown to negatively affect grassland yields and biomass production (Environment Agency, 2006; Niu et al., 2014). Recent spatial analysis of long-68 term temperature and precipitation records, in combination with land use data, highlighted an 69 increasing risk of flooding and drought (single and in combination/succession) in many UK 70 pastoral landscapes (Dodd et al., 2021). To safeguard the vital ES provided by agricultural 71 grasslands, we need to fully understand the impact of such events on the whole ecosystem. 72 Precipitation regimes regulate grassland ecosystem structure and function due to the 73 74 influence on soil moisture, a major driver of plant growth and microbial activity (Bloor and 75 Bardgett, 2012). While there is some uncertainty in the response pattern (Evans et al., 2022), plants and microbes are considered to exhibit a hump-shaped response to moisture with 76 substrate diffusivity and oxygen limitation constraining growth at the low and high extremes 77 78 respectively. Consequently, extreme events which rapidly and dramatically change the moisture conditions (such as flood and drought) have the potential to cause a large-scale 79 change in above and below ground ecosystem processes. A wealth of research on drought has 80

demonstrated large scale changes in ecosystem properties including reductions in primary 81 production (Ciais et al., 2005; Hoover et al., 2014), changes in above and below ground 82 83 community structure (de Vries et al., 2018; Knapp et al., 2020; Ochoa-Hueso et al., 2020; Ochoa-Hueso et al., 2018) and altered C and nutrient cycling (Bloor and Bardgett, 2012; 84 Deng et al., 2021; Dijkstra et al., 2015). However, research into ecosystem responses to 85 prolonged flooding at the field scale have been largely neglected, and while some 86 87 experiments include increased precipitation regimes, these rarely examine prolonged flooding (Abbasi et al., 2020), despite evidence of widespread impacts on vegetation across the globe 88 89 (Famiglietti et al., 2021). Flooding disruption of nutrient cycles (Sánchez-Rodríguez et al., 2019a,b) and increased N<sub>2</sub>O emissions (Oram et al., 2021; Sánchez-Rodríguez et al., 2019a; 90 Sánchez-Rodríguez et al., 2019b) along with changes in microbial communities (Sánchez-91 92 Rodríguez et al., 2019a,b; Unger et al., 2009) have been observed in mesocosm studies under controlled conditions. However, such studies may exhibit differing responses to the field 93 scale (Unger et al., 2009). Alternatively, flood impacts have been assessed following natural 94 flood events on existing field trials (González Macé et al., 2016; Harvey et al., 2019; Wagner 95 et al., 2015). Nevertheless, these opportunistic trials often have limited ability to regulate the 96 experimental conditions and are often poorly instrumented and lack appropriate controls and 97 plot scale flood experiments remain a key gap in the literature. 98

In addition to single extreme events, there is growing recognition that many ecosystems can experience a combination of multiple climatic drivers or hazards often termed compound extremes (Zscheischler et al., 2018). Such compound events are likely to have a larger effect on ecosystem function, through a reduction in the systems resilience, amplifying the impact and potentially leading to bi-modal response-recovery patterns indicative of a regime shift (Rillig et al., 2019). While research into the impact of compound events on ecosystems is growing, these have been mainly focused on combinations of drivers with additive properties,

for example heatwaves and droughts (Arain et al., 2022) or storm surge and flooding (Wahl
et al., 2015). However, recent research suggests that the occurrence of multiple extreme
events, with contrasting drivers, within the same year is also increasing; for example a swift
transition from flood to drought events or *vice versa* (Dodd et al., 2021).

As ecosystem function is highly dependent on soil moisture, contrasting events of 110 extreme precipitation amount, occurring in quick succession, are likely to have a different 111 112 impact to each event alone. A deluge event during summer drought was seen to alleviate reductions in plant production and soil respiration in a natural semi-arid grassland (Post and 113 114 Knapp, 2020) and numerous studies have examined the effect of rewetting of dry soils on many aspects of ecosystem function (Birch, 1958; Borken and Matzner, 2009; Fierer and Schimel, 115 2002; Gordon et al., 2008). However, there has been little investigation of the reverse, where 116 rapid drying of wet soils may occur due to reduced rainfall. The frequency of winter and spring 117 flooding and that of summer droughts is predicted to increase (Fowler and Kilsby, 2003; 118 Thompson et al., 2017). Furthermore, as extreme events are becoming more frequent and 119 intense (Li et al., 2019; Trenberth, 2011), the likelihood of a second extreme occurring before 120 the system has fully recovered also increases, potentially magnifying the impact (Schwalm et 121 al., 2017) and tipping the system to a new functional state. We propose two alternative 122 hypotheses of ecosystem response to this scenario: (A) the drawdown of soil moisture may be 123 slower in previously flooded soils promoting resilience to subsequent drought and a reduction 124 125 in observed ecosystem impact (Post and Knapp, 2020), or (B) if the drought event occurs prior to ecosystem recovery, a second stress event may lead to adverse impacts on biotic 126 communities and potential community collapse, with multi-year impacts on ecosystem 127 function. 128

Our study directly addresses gaps in knowledge surrounding the short and medium-term impact of simulated severe drought and flood events on the resilience of grassland

ecosystems at the field plot scale. Further, we assess the impact of compound weather events 131 characterised by a spring flood followed by a summer drought. Here, we use a set of above 132 133 and below ground biotic and abiotic indicators to quantify the effect of these extreme events on ecosystem process of six essential grassland ES: forage production, nutrient cycling, 134 organic matter decomposition, climate regulation, pollution regulation and biodiversity 135 provision. Changes in these indicators were used to assess the impact of each event type 136 137 immediately post stress (reflecting system resistance) and the legacy implications for longerterm ecosystem function through monitoring indicator recovery (reflecting system resilience). 138 139 We hypothesised that (1) extreme weather events will negatively impact on ecosystem processes; (2) grassland ecosystems will have different degrees of resistance and resilience to 140 flood or drought stress. Additionally, we investigated the two alternative hypotheses 141 presented above (A and B) for the combined flood and drought event. 142

143

#### 144 **2. Materials and methods**

#### 145 2.1. Treatments and plot establishment

146 The study site was located at the Henfaes Agricultural Research Station,

147 Abergwyngregyn, Gwynedd, North Wales, UK (53°14′21"N, 4°00′57"W) on sheep-grazed,

148 *Lolium perenne* L. dominated, low intensity grassland. The soil at the site is a sandy loam

textured Eutric Cambisol (Typic Hapludalf) overlying a mixed glacial till parent material.

150 The site has a temperate-oceanic climate with a mean annual rainfall of 1060 mm and

temperature of 10 °C.

152 Sixteen 3 m  $\times$  3 m plots were established in winter 2015. The experiment consisted of four

- treatments and was conducted in three phases. The treatments consisted of (1) spring flood
- 154 (flood), (2) summer drought (drought), (3) spring flood followed by summer drought
- 155 (flood+drought), and (4) ambient conditions (control). Each treatment had four replicates (n =

4) arranged in a randomised plot design. The three data collection phases were (1) floodperiod, (2) drought period and (3) recovery.

158 Although the site was previously grazed with sheep (*Ovis aries* L.), livestock were 159 excluded from the plots during the construction phase. Two weeks prior to the start of the 160 trial (April 2016), sheep were briefly reintroduced (0.22 sheep  $m^{-2}$ ; 2 d) to mimic mob 161 grazing and trampling in this sheep-based pasture system.

To prevent lateral water escape, the eight flood plots were hydrologically isolated.
This was achieved by vertically inserting 80 cm deep PVC plastic boards (5 cm thick; TatraRotalac Ltd., Manchester, UK) lined with an impermeable butyl rubber membrane
(Pondkeepers Pond Liners Ltd., Billingham, UK; Fig. S1) around the plot boundary. The
boards were buried to a depth of 30 cm in the soil, leaving 50 cm aboveground to hold the
flood water. A physical barrier was also inserted to a similar depth to isolate the eight nonflood plots to ensure a similar level of physical disturbance.

To mimic on-farm management practices, lime (3 t ha<sup>-1</sup>) and NPK compound fertiliser (25:5:5; 50 kg N ha<sup>-1</sup>) were applied to all plots in April 2016 (14 d prior to flooding), in line with past fertiliser application rates in this field. The same rate of NPK fertiliser was applied again in August 2016 during the drought period.

173

174 *2.2. Experimental details* 

The flooding treatments were designed to simulate a fluvial flood event, reflecting the scale and duration of similar events which have occurred within the local and wider region in the last decade (Defra, 2014; Harvey et al., 2019; Huntingford et al., 2014; Slingo et al., 2014). However, it should be noted that the site had no previous history of flooding. Figure S1 shows the layout of the field trial.

180 <u>Flood phase</u>: In spring 2016, eight flood plots were submerged with water from the 181 adjacent river to a depth of 20 cm, which was sufficient to fully submerge all vegetation (Fig. 182 S1.c). Sediment was added to each of the plots following the initial flooding to a depth of 2-3 183 mm to simulate the deposition of eroded soil and sediment typically associated with river 184 flooding and observed in recent extreme flood events (ADAS, 2014). This was achieved by 185 suspending 13.5 kg of surface soil in the floodwater. The soil was taken adjacent to the plots 186 (0-7 cm depth) under the same management regime.

187 The plots were maintained at a constant flood level of 20 cm for eight weeks during 188 April to June, by topping up the flood water, via a ball float valve connected to a reservoir of 189 river water, reflecting the unprecedented flooding events observed across UK agroecosystems 190 in the winter of 2013 - 2014 (Defra, 2014; Slingo et al., 2014). At the end of the flood period 191 the floodwater was removed using a pump over a 4 h period.

192 <u>Recovery phase 1:</u> All plots were left under ambient conditions for four weeks193 following the end of the flood phase and before the initiation of the drought phase.

Drought phase: At the end of recovery phase 1, rain-out shelters ( $4 \text{ m} \times 4 \text{ m}$  area) 194 were erected over eight plots, four of which were previously subjected to the spring flood. 195 The rain-out shelters were constructed with PalSun<sup>®</sup> polycarbonate sheets (2 mm thick; 196 Plastock Ltd, High Wycombe, UK), mounted on wooden frames, angled towards the 197 direction of the prevailing wind (Fig. S1d) with a maximum height of 1.8 m. The slight 198 199 reduction  $(10 \pm 1\%)$  in light transmission under the rain-out shelters (measured using a PAR sensor; 400 to 750 nm; PP Systems International Inc., Amesbury, MA) was deemed unlikely 200 to impact significantly on grass growth, during the drought treatment. Rain was excluded 201 202 from the four drought and four flood+drought plots for 8 weeks during July to September 2016. 203

204 <u>Recovery phase 2</u>: All plots were monitored for two years following the end of the 205 drought period to determine the impact on ecosystem recovery in the absence of management 206 intervention. During this time, all plots were subjected to ambient conditions and livestock 207 and rabbits were excluded.

208

### 209 2.3. Plot measurement frequency and ecosystem service assessment

210 To determine the impact on ES, a range of above and below ground indicators were identified which are directly linked to the provision of the six identified ecosystem services, 211 212 forage production, nutrient cycling, organic matter decomposition, climate regulation, pollution regulation and biodiversity provision (Table 1; Adhikari and Hartemink, 2016; 213 Dominati et al., 2010; Rutgers et al., 2012; van Eekeren et al., 2010). Additionally, soil biota 214 play a key role regulating the ecosystem processes underpinning these ES (Creamer et al., 215 2022). The importance of soil biodiversity in ecosystem multifunctionality is becoming 216 increasingly apparent (Creamer et al., 2022; Delgado-Baquerizo et al., 2020) along with the 217 role of plant-soil interactions (Forero et al.; Valencia et al., 2018). As such, the following 218 above and below ground biotic indicators supporting ecosystem processes were also 219 included: pasture community composition, microbial community characteristics (biomass and 220 PLFA), and earthworm abundance and biomass. Each plot was divided into dedicated areas 221 for monitoring (Fig. S1). During the flood, drought and recovery phases regular 222 223 measurements were taken from all plots (see Table S1).

224

### 225 2.4. Above ground measurements

Biomass was harvested from  $40 \times 40$  cm quadrats within each plot on 5 occasions (1 month post flood, 1 week post drought, and at 6-, 12- and 24-months recovery). Plant material was oven dried (80 °C, 24 h), weighed, ground (< 2 mm) and analysed for forage quality

including crude protein, metabolizable energy and digestibility (Sciantec Analytical
Laboratories, York, UK). Annual biomass yield was calculated as tonnes per hectare for the
four treatments as the sum of the first 4 cuts for year 1 and as the 5<sup>th</sup> cut for year 2.

Plant surveys were undertaken over each entire plot by the same expert botanist in the growing seasons throughout the duration of the experiment at the same time as the biomass harvest. Presence and percentage cover (rounded to the nearest 1%) of all vascular plants and bryophytes were recorded in each experimental plot. Cover of bare ground plus litter and total bryophyte were also recorded and the species richness and Shannon-Wiener Diversity were calculated (Magurran, 2013).

The percentage cover of each species was converted to a fraction. The proportion of forage grasses which directly support livestock grazing and hence food production and injurious weeds which detract from this provision (Maskell et al. 2020) were calculated along with indicators of important species supporting biodiversity, namely; butterfly larval food plants; supporting invertebrate populations (Lepidoptera) (Smart et al., 2000; Smart et al., 2017); crop wild relatives as genetic insurance for food production (Jarvis et al., 2015) and nectar-providing plants supporting pollinator biodiversity (Baude et al., 2016).

245

#### 246 2.5. Below ground measurements

The plots were instrumented with SDI-12 soil moisture sensors (Acclima Inc.,
Meridian, ID) inserted horizontally at a depth of 5 cm, a static greenhouse gas chamber and
three MacroRhizon soil solution samplers (0.15 µm pore size; Rhizosphere Research
Products, Wageningen, The Netherlands) inserted at a 45° angle to a depth of 5 cm for
collection of soil pore water with two inserted within the main plot and one within the
greenhouse gas chamber. Soil sampling was undertaken immediately following the removal
of the flood or drought stress and at 6-, 12- and 24-month post drought recovery stages.

During each soil sampling event multiple soil cores (n = 6,  $\phi = 1$  cm, depth = 0-10 cm) were randomly taken from the indicated plot area (Fig. S1), homogenised and sieved (< 2 mm) for analysis.

Soil physical indicators: An intact core (100 cm<sup>3</sup>, 0-10 cm depth) was taken from each
plot and weighed, dried (105 °C, 16 h), reweighed and dry bulk density and water filled pore
space determined.

260 Soil (and floodwater) chemical indicators: Soil moisture content was determined gravimetrically (105 °C, 24 h). Soil pH and electrical conductivity (EC) were measured in 1:5 261 262 (w/v) soil to distilled water suspension using standard electrodes. Within 24 h of collection, soils were extracted with 1:5 (w/v) soil-to-AcOH (0.5 M) and 1:5 (w/v) soil-to-K<sub>2</sub>SO<sub>4</sub> (0.5 263 M). Macronutrient and trace element (P, K, Ca, Na, Al, Fe, Mg, Mn, Al) concentrations were 264 determined in the AcOH extracts via ICP analysis (Varian 720 ICP-OES). NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in 265 the K<sub>2</sub>SO<sub>4</sub> extracts were measured colorimetrically according to Miranda et al. (2001) and 266 Mulvaney (1996), respectively. 267

Total soil C and N were determined on oven-dried, ground soil using a TruSpec CN Analyser (Leco Corp. St. Joseph, MI, USA). The tea-bag index (TBI) was used as a standardised method to estimate both C decomposition (*k*) and C stabilisation (*S*) rate in soil (Keuskamp et al., 2013). The difference between the two rates provides an indication of C storage. Tea-bags were buried at 5 cm depth within each plot immediately prior to stress initiation (Keuskamp et al., 2013) and recovered at the end of each stress period.

Soil redox measurements were taken on a weekly basis during the experiment with a handheld SenTix<sup>®</sup> redox probe ( $n = 3 \text{ plot}^{-1}$ ; Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) inserted into the top 2 cm of soil. During the flood phase, soil solution and the overlying floodwater was sampled weekly from the flood plots. Where possible, the soil solution was sampled from the control treatment, however, there was only

sufficient soil moisture to extract a sample on limited sampling dates. These samples were
analysed for pH, EC, NH4<sup>+</sup> and NO3<sup>-</sup> as described above and dissolved organic C (DOC)
using a Multi N/C 2100-S Analyser. Dissolved reactive P (DRP) and total dissolved P (TDP),
after acid persulfate digestion (Rowland and Haygarth, 1997) were determined using the
molybdate blue method of Watanabe and Olsen (1965). Dissolved organic P (DOP) was
calculated as the difference between TDP and DRP (AnalytikJena, Jena, Germany).

Soil biological indicators: Following collection, soil samples were immediately
frozen (-80 °C), freeze-dried and phospholipid fatty acid (PLFA) analysis undertaken
according to Bartelt-Ryser et al. (2005) to determine the soil microbial community profile.
Taxonomic groups were ascribed to individual PLFAs using the Sherlock<sup>®</sup> PLFA Method
and Tools Package (PLFAD1: Microbial ID Inc., Newark, DE) as outlined in SánchezRodríguez et al. (2019b).

Earthworm surveys were undertaken at the same timepoints as for soil sampling, by excavating a soil pit (20 cm × 20 cm × 20 cm) and recovering live earthworms by hand. Following soil excavation, 1 L of allyl isothiocyanate (1.3% v/v) in deionised water was poured into the pit and left for 30 min to expel any deeper dwelling earthworms (Pelosi et al., 2009). Collected earthworms were transported live to the laboratory in moist soil where numbers of juvenile and mature earthworms were recorded and weighed.

*Greenhouse gas emissions:* A closed static chamber  $(40 \text{ cm} \times 40 \text{ cm} \times 25 \text{ cm})$  was fitted to an aluminium frame installed in each plot at the beginning of the field experiment. Immediately after closing each static chamber, a 20 ml gas sample was taken from the headspace through a septum inserted in the lid using a needle attached to a 25 ml syringe at time T0 and T60 mins. The gas samples were transferred to pre-evacuated glass vials (20 ml) and analysed for CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>O using a Clarus 500 gas chromatograph equipped with a HS-40 Turbomatrix autoanalyzer (PerkinElmer Inc., Waltham, MA).

Gas sampling was repeated 40 times between the start of the flood phase to day 235, 304 for each plot when no differences in gas flux had been observed between the treatment and 305 control plots for 90 days The frequency of gas sampling was higher during the flood phase, 306 recovery phase 1 and the drought phase, and lower in recovery phase 2. At each gas sampling 307 time, soil temperature was recorded (0-2 cm depth) (T0 and T60 min) and the temperature 308 was used to correct the GHG fluxes. GHG fluxes were calculated using the difference in each 309 310 gas concentration between T0 and T60, the ratio between chamber volume and soil surface area (Chadwick et al., 2014), and based on tests showing linearity (MacKenzie et al., 1998). 311 312 During the flood phase, an extension (25 cm height) was used to extend the static chamber in the flood plots. Total cumulative fluxes were estimated using the trapezoidal rule (Rahman 313 and Forrestal, 2021) for the different phases of the experiment; the flood phase (0 to 58 d), 314 the flood and recovery phase 1 (0 to 85 d), the drought phase (93 to 142 d), the drought and 315 recovery phase 2 (93 to 142 d), and the whole period of gas sampling (0 to 235 d). The total 316 GHG flux in CO<sub>2</sub> equivalents (kg  $C_{eq}$  ha<sup>-1</sup>) was calculated by multiplying the total 317 cumulative fluxes of CH<sub>4</sub> by 28, CO<sub>2</sub> by 1, and N<sub>2</sub>O by 265 (IPCC, 2013) and summing 318 319 them.

320

321 2.6. Data processing and statistical analyses

322 Soil moisture data from the Acclima moisture probes was converted to water filled pore323 space (WFPS) as follows:

324 
$$WFPS(\%) = \frac{SWC}{\left(1 - \frac{BD}{PD}\right)} \times 100$$
 (Eqn. 1)

where SWC = volumetric soil water content (vol. %), BD = soil bulk density (g cm<sup>-3</sup>) and PD = particle density (2.65 g cm<sup>-3</sup>).

To examine the response of the above- and below-ground metrics to the treatments, natural log response ratios (*lnRR*) were calculated as follows (Hedges et al., 1999):

329 
$$lnRR = ln\left(\frac{X_{treatment}}{X_{control}}\right)$$
 (Eqn. 2)

where  $X_{treatment}$  and  $X_{control}$  are the mean values of each indicator under the treatment (flood, drought and flood + drought) and the ambient control plots. In accordance with (Hedges et al., 1999), we calculated the variance as follows:

333 
$$Variance = (SD_{treatment}^2 / (nX_{treatment}) + (SD_{control}^2 / (nX_{control}))$$
(Eqn. 3)

334 Subsequently the variance was converted to a 95 % confidence as shown below:

335 *Confidence interval* = 
$$1.96 x \sqrt{Variance}$$
 (Eqn. 4)

Finally, the response ratio and the corresponding confidence intervals were transformed to apercentage change of the indicator from the control as follows:

 $(e^{lnRR \, or \, Variance} - 1) \times 100 \tag{Eqn. 5}$ 

The percent change and associated confidence interval was calculated for each above- and below-ground indicator and the result plotted for three time periods: (1) immediately post stress treatment; (2) after 1 year recovery; and (3) after 2 years recovery. If the 95 % confidence interval did not overlap with zero, this indicated a significant change in the indicator metric.

Changes in soil microbial communities were assessed by principal component analysis (PCA) of PLFA taxonomic groups based on a data correlation matrix with principal components (PCs). Analysis of variance (ANOVA) with Tukey's HSD post-hoc testing was used to assess differences in the four treatments (for plant food production and biodiversity provision plant indicators and greenhouse gas fluxes for each of the identified time periods). The statistical cut-off for significance was p < 0.05. ANOVA and PCA were performed in the statistical package SPSS software v22.0 (IBM Inc., Armonk, NY).

#### 352 **3. Results**

353 *3.1. Assessment of treatment impacts* 

Measurements of WFPS were used to validate the success of the treatments imposed 354 on the plots. At the initiation of flooding, WFPS increased from 34 % to a maximum of 85 %, 355 and remained at  $79 \pm 1$  % for the duration of the flood period. The control plots fluctuated 356 between 20 and 45 % WFPS during the same period (Fig. S2). The drought treatments 357 resulted in a 71 % reduction in WFPS from 32 %, reaching 9 % by the end of the drought 358 period which was 63 % lower than the control. While the % reduction in WFPS over the 359 360 drought period was similar in the previously flooded plots, exhibiting a 70 % reduction from 54 % to 16 %, due to a higher initial value, this was only 47 % below the control. Both 361 362 flooding and drought exhibited a legacy effect from the stress, slowly converging toward the control. The WFPS remained elevated in the flood plots and reduced in the drought plots, 363 compared to the control for the 6 months post drought when the sensors were removed. In 364 contrast, the flood+drought treatments showed a quicker recovery in WFPS following 365 drought treatment and WFPS was comparable to the control plots within 2 months (Fig. S2). 366 Flooding reduced the redox potential (Eh) from +297 mV (aerobic) to -92 mV 367 (anaerobic) after 50 d of flooding (Fig. S3), while in the control and drought plots Eh values 368 remained between +200 and +300 mV, throughout the trial. On removal of the flood water, 369 Eh rapidly increased to a maximum of 348 mV in the first 14 days and remained higher than 370 the control for 28 days before returning to the control range (+200 to +300 mV). While no 371 clear trends emerged during the drought phase all treatments plots showed a general trend of 372 373 higher Eh compared to the control during recovery 2 in the order of flood > flood+drought > drought > control. 374

Neither flooding nor drought led to a change in soil temperature. In addition, no
unexpected periods of flooding or drought occurred during the experiment which might have
compromised the control treatments.

378

#### 379 *3.2. Above-ground indicator metrics*

The response of each above-ground indicator immediately following the stress period 380 381 and following the three recovery periods are summarised in Figure 1. The annual aboveground plant biomass for the flood plots was reduced by 30 % (CI: 24 %) compared to the 382 control, while the drought treatment did not significantly impact annual biomass production 383 (Fig. 1b). The reduction in annual biomass in flood+drought plots was greater than for flood-384 385 only (54 % decrease, CI: 30 %). However, these changes in biomass were not carried over to the following year (Fig. 1c). The change in pasture community which established post flood 386 387 (Table S2a) was accompanied by a change in the plant species supporting food provision and biodiversity ES. The first month's pasture regrowth following flood stress showed a reduced 388 abundance of forage grasses, crop wild relatives (CWR) and plant species known to provide 389 390 food for butterfly lava (Fig. 2). There was also a slight increase in the number of tree and shrub species seedlings (comprised of Fraxinus excelsior) and an increase in the number of 391 nectar-producing species. The reduction in forage grasses, CWR and butterfly lava food 392 remained for the first-year post flood, and the abundance of CWR and butterfly lava food 393 species remained reduced in the second year of recovery. Immediately following the drought, 394 the previously flooded plots continued to show a reduction in forage grasses, CWR and 395 396 butterfly lava food species. Additionally, these plots showed a small reduction in legumes. The reduction in CWR and butterfly lava food species was still evident in the first year along 397 398 with an increase in the nectar species, but these changes were not present in the second year of recovery. In contrast, post drought there was no change in these indicator species in 399 drought-only plots across the whole trial (Fig. 2, Table S2b). 400

401

402 *3.3. Below-ground indicator metrics* 

The response of below-ground soil quality indicators to flooding and drought are summarised in Figure 1. Overall, significant changes in a range of soil quality indicators were apparent immediately post-stress removal, however, these changes progressively lessened over the 2-year recovery period. In addition, the response of the combinatorial flood+drought treatment was similar to the flood treatment.

408

#### 409 *3.3.1. Soil chemical indicators*

Flooding reduced soil EC, soil available P ( $P_{acOH}$ ), and C storage compared to the control (Fig. 1a), and increased extractable NH<sub>4</sub><sup>+</sup>, Mn (Mn<sub>acOH</sub>), and Fe (Fe<sub>acOH</sub>). Drought also reduced C storage and increased extractable NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and Fe<sub>acOH</sub> along with the soil EC. The flood+drought reduced available P ( $P_{acOH}$ ) and increased soil EC, Mn<sub>acOH</sub>, Fe<sub>acOH</sub>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>.

During the first year of recovery PacoH was reduced in all stress combinations (Fig. 415 1b) but was increased compared to the control in the second-year post stress in the flood and 416 flood+drought plots (Fig. 1c). Extractable FeacOH and MnacOH concentrations were increased 417 in the flood plots and Mn<sub>acOH</sub> increased in the flood+drought plots during the first year. Two 418 years post stress Fe<sub>acOH</sub> concentrations were reduced in both treatments. Flooding increased 419 soil NH4<sup>+</sup>, and drought increased both soil NH4<sup>+</sup> and NO3<sup>-</sup> but this did not persist through the 420 recovery phase. However, at the two-year recovery point NO<sub>3</sub><sup>-</sup> concentrations were increased 421 422 in the flood plots.

Due to low moisture conditions in some treatments, soil solution samples were only consistently collectable from the flood plots, and only during the flood phase and recovery phase 1. There was a general trend of increasing DOC and decreasing DRP and  $NO_3^-$  as the flood period progressed (Fig. 3). On removal of the flood water there was a spike in DOC concentrations accompanied by a spike in DOP followed by a sharp decline. Additionally,

there was a large spike in the NH4<sup>+</sup> concentrations 13 d following removal of the flood water
to a maximum of 131 mg N L<sup>-1</sup> which quickly declined to background levels (0.04 mg N L<sup>-1</sup>)
by day 21. A sharp spike in NO<sub>3</sub><sup>-</sup> concentrations to a maximum concentration of 34.9 mg N
L<sup>-1</sup> was observed 7 days after flood removal followed by a more gradual decline to
background levels (0.83 mg N L<sup>-1</sup>) by day 27.

433

434 *3.3.2. Soil biological indicators* 

Distinct changes in microbial community structure were observed with respect to both 435 436 sampling time and treatment. Seasonality played a key role in controlling the distribution of PLFA markers with marked separation between the stress and recovery periods and a clear 437 shift towards more Gram-positive dominated communities as time progressed. However, 438 treatment effects on community structure were also observed (Fig. 4). Flood stress increased 439 the dominance of Gram-negative bacteria while drought led to a shift towards actinomycete 440 markers. The flood and flood+drought plots also had a reduction in the fungi-to-bacteria ratio 441 and amount of arbuscular mycorrhizal fungi (AMF) in the first- and second-year post stress 442 (Fig 1.b.c). The PLFA markers within the flood plots showed a larger separation from the 443 control treatments compared to the drought and flood+drought plots after the first and second 444 year of recovery. After 1-year of recovery there were three distinct groups of PLFA 445 distributions, (1) control, (2) drought and flood+drought, and (3) flood. At the 2-year 446 447 recovery point the control group remained separated from the three stress treatments (Fig. 4). The size of the microbial biomass was also slightly increased in the flood and drought plots 448 during the first year and in the flood-only and flood+drought plots in the second year relative 449 to the control. Earthworm abundance increased following removal of flooding, however, it 450 decreased following the removal of drought (Fig. 1). 451

452

#### 453 *3.3.3. Greenhouse gas emissions*

During the flood phase, CO<sub>2</sub> emissions were higher in the control plots in comparison 454 with the flood plots (up to 300 mg C m<sup>-2</sup> h<sup>-1</sup> vs. <1 mg C m<sup>-2</sup> h<sup>-1</sup>, respectively; Fig. 5b). 455 During the drought CO<sub>2</sub> emissions were similar for the control and flood plots and reduced in 456 the drought and flood+drought plots (between 80-320 mg C m<sup>-2</sup> h<sup>-1</sup> vs. 30-190 mg C m<sup>-2</sup> h<sup>-1</sup>, 457 respectively; Fig. 5b). During recovery phase 1 and 2, the CO<sub>2</sub> emissions were similar 458 459 between all treatments. Overall, flood or drought caused a large decrease in cumulative CO<sub>2</sub> emissions while their combination (flood+drought) resulted in the lowest cumulative 460 461 emissions (Table 2).

No N<sub>2</sub>O emissions were observed during the flood phase for the control or flood plots. 462 During the drought phase, application of N fertiliser resulted in a peak in N<sub>2</sub>O emissions in 463 the control and flood plots (up to 0.43 and 0.82 mg N m<sup>-2</sup> h<sup>-1</sup>, respectively) while no 464 emissions were observed in the drought or flood+drought plots (<0.07 mg N m<sup>-2</sup> h<sup>-1</sup>). The 465 spring flood caused N<sub>2</sub>O peaks of 0.2–1.2 mg N m<sup>-2</sup> h<sup>-1</sup> (60-85 d after flooding) that were 466 more frequent and higher than the fluxes observed following N fertilization in the control 467 (Fig. 5c). No N<sub>2</sub>O emissions were observed in any of the plots in the second recovery phase 468 (Fig. 5c). The highest total cumulative N<sub>2</sub>O emissions were calculated for the flood+drought 469 plots (4.16 kg N ha<sup>-1</sup>), followed by flood plots (3.35 kg N ha<sup>-1</sup>), control plots (1.01 kg N 470  $ha^{-1}$ ) and drought plots (0.41 kg N  $ha^{-1}$ ); the differences were significant between the 471 472 flood+drought and the drought plots (Table 2). These emissions occurred mainly during recovery phase 1 (Table S3). The expected increase in N<sub>2</sub>O emissions due to N fertilisation 473 (during the drought phase) was influenced by the treatment where total cumulative N<sub>2</sub>O 474 emissions followed the order flood  $\geq$  control = flood+drought  $\geq$  drought (Table S3). 475 CH<sub>4</sub> emissions remained close to zero except immediately after flood water removal 476 (58 d after flooding) when they peaked (1-2 mg C  $m^{-2} h^{-1}$ ) in the flooded plots (Fig. 5a). 477

Mean cumulative CH<sub>4</sub> fluxes were small and not significant between treatments over the 2-478 year experiment (Table 2). Overall, the control and drought treatments acted as a sink for 479 CH<sub>4</sub> (-0.38 and -0.20 kg C ha<sup>-1</sup>, respectively) while the flood and flood+drought were a 480 minor source of CH<sub>4</sub> (0.98 and 1.49 kg C ha<sup>-1</sup>, respectively). Table S3 shows that (i) the 481 control plots were a sink during most of the experiment (except in the second recovery 482 phase), (ii) flood induced CH<sub>4</sub> emissions (mainly immediately following flood water 483 484 removal), and (iii) drought reduced CH<sub>4</sub> emissions (only during the drought phase). When considering the combined emissions of GHGs in terms of total CO<sub>2</sub>-485 486 equivalents, significant differences were apparent and followed the series control  $\geq$  flood  $\geq$ drought = flood+drought (Table 2). If direct  $CO_2$  emissions are excluded, the pattern was 487 similar to total cumulative N<sub>2</sub>O emissions (i.e. flood+drought  $\geq$  flood  $\geq$  control  $\geq$  drought) 488 over the 2-year period. 489

490

### 491 **4. Discussion**

This study utilised a suite of above and below ground properties of an improved sheep 492 grazed pasture to determine the impact of both single extreme events characterised by 493 contrasting rainfall patters (flood or drought), and the combined impact of a swift transition 494 495 from flood to drought conditions on ecosystem functioning. Our results clearly show three key findings: (1) this grassland system was more resistant and resilient to drought conditions 496 than flooding, (2) the combination of flood and drought did not magnify the impact of the 497 single stressors with some indication of faster recovery times and (3) persistent changes in 498 plant and microbial communities post flood likely have implications for future ecosystem 499 service provision. 500

501

502 *4.1. Impact of contrasting single extreme events (i.e. flood vs. drought).* 

Both flooding and drought were found to have a large impact on the above and below 503 ground indicators of ecosystem function in improved grasslands supporting out hypothesis 504 505 that extreme weather events will negatively impact ecosystem processes. However, flooding was more detrimental with 17 indicators being different to the control immediately post stress 506 compared to 8 indicators for drought (Fig. 1). Flood plots also showed a difference in 7 507 indicators two years after the stress compared to just 1 indicator two years post drought. 508 509 Together these results show lower resistance and resilience to flooding than drought. Further evidence of this was found in the shifts in microbial community, as indicated by 510 511 PLFA markers. Larger shifts occurred for the flood plots and remained more distinct for longer compared to drought. This prolonged shift in microbial communities agrees with 512 previous mesocosm studies, where a shift in PLFA markers occurred following extreme 513 flooding (80 days) and persisted for the length of the monitored recovery period of 60 days 514 (Sánchez-Rodríguez et al., 2018). In contrast, microbial communities in grasslands have been 515 shown to be largely resistant to drought (Birkhofer et al., 2021; Blankinship et al., 2011) and 516 unlike for flooding there was no change in microbial biomass during the drought period 517 Drought did induce a larger shift towards actinomycete taxa which have been shown to be 518 more resistant to dry and wetting cycles (Acosta-Martínez et al., 2014; Ochoa-Hueso et al., 519 520 2020; Ochoa-Hueso et al., 2018). However, seasonality had a large control on PLFA marker distribution likely due to changing environmental conditions. Interestingly, the shift towards 521 522 actinomycetes compared to the control also occurred in the flood plots post drought, despite no drought treatment being imposed. This indicates that immediately following 523 environmental stress, microbial communities are more responsive to changes in 524 environmental conditions compared to unstressed communities demonstrating lower 525 resilience. 526

Environmental stressors can have detrimental impacts on earthworm communities 527 (Singh et al., 2020). Earthworms are considered keystone species within soil environments 528 529 due to their influence on soil chemical, physical and biological processes (Blouin et al., 2013; Jones et al., 1994). Generally, prolonged flooding has been shown to reduce earthworm 530 abundance across different ecosystems (Singh et al., 2020). However, our study showed a 76 531 % increase in earthworm numbers (predominantly juveniles) immediately following removal 532 533 of the overlying floodwater. Visual observation suggested this was the result of recent hatching, with some earthworms still attached to cocoons. This finding has only been 534 535 reported for one study in annually flooded Alder forests (Emets, 2018). We hypothesise that a sudden change in osmotic potential ruptured the cocoons outer shell leading to premature 536 emergence. In contrast drought did not impact on earthworm biomass or abundance, likely 537 due to their ability to enter estivation in response to low moisture conditions, indication of 538 which was observed in the post drought sampling with many individuals exhibiting 539 characteristic knot configurations (McDaniel et al., 2013). The trend post flood did not persist 540 into the recovery phase once cocoons within the control soils hatched, and under the single 541 flood stress condition this short-lived increase in earthworm numbers is unlikely to influence 542 overall ecosystem function. 543

Above and below-ground biodiversity has been shown to be positively related to 544 ecosystem multifunctionality (Delgado-Baquerizo et al., 2020) and even small changes in 545 microbial structure (such as community composition shifts) can have a large impact on 546 function due to altered metabolic capability of the ecosystem (Crowther et al., 2019). The 547 pronounced and persistent change microbial communities induced by flooding were also 548 accompanied by increased plant diversity and richness which will influence soil function with 549 knock-on effects on the provision of ecosystem services while the impact of drought appears 550 to be minimal and immediate with limited longer-term impacts. Where significant indicator 551

responses to treatments were found, their impact on the 6 highlighted ES will be discussed insection 4.3.

554

4.2. Impact of consecutive contrasting extreme event types (i.e. flood vs. drought).

The combination of flood and drought stress did not increase the number of indicators 556 affected compared to flood alone either immediately post stress (15 for flood+drought cf. 17 557 558 for flood) or at the 2-year recovery (5 flood+drought cf. 6 for flood). In fact, there was some indication within the microbial community of faster recovery in community composition 559 560 evidenced by reduced separation of PLFA markers from the control plots 1 year post stress compared to flood alone. We suggest that the more rapid reduction in soil moisture post flood 561 in the flood+drought plots may have increased the resilience of microbial population in line 562 with the first of the two alternative hypotheses that subsequent events of contrasting typology 563 will promote ecosystem recovery (hypothesis A). 564

However, while hypothesis A may fit the microbial community response the impact 565 on earthworm populations showed contrasting results. The large increase in juvenile 566 earthworm numbers post flood was followed by a dramatic decrease of 85 % in abundance 567 compared to the control post drought despite no significant change in the drought plots. We 568 suggest that the premature emergence of juveniles during flooding may have implications for 569 570 the resilience of the community if a second extreme event occurs prior to this community 571 reaching maturity, in line with the second alternative hypothesis B. Larger soil invertebrates including earthworms are especially important for maintaining optimal ecosystem functions 572 (Delgado-Baquerizo et al., 2020) and loss of earthworm populations could have large-scale 573 consequences for ecosystem service provision. 574

575

576 *4.3 Implications for ecosystem service provision* 

Flood, drought and the combined flood+drought had an impact on both the indicators directlyrelated to the 6 specific ecosystem services considered.

579 *Forage production*: The reduction in annual biomass and the slow recovery of forage grass species following flooding will likely decrease production at least during the event year. 580 Additionally, flooding resulted in large periods of increased bare ground while the plant 581 community re-established itself. The extremes of wet and dry conditions will limit the pool of 582 583 successful colonists. A likely scenario in the longer term is larger areas of bare ground with varying cover of transient and persistent weed species. Recolonisation of bare ground with 584 585 invasive weed species is a concern to farmers (ADAS, 2014). However, in our study neither flooding nor drought or the combination led to a change in the % cover of injurious weeds 586 compared to ambient conditions. 587

These results indicate that flooding will lead to a reduction in forage provision and 588 subsequent production metrics at least during the stress year while drought may have limited 589 impact in this grassland. Farmers may need accept reductions in profit margins to bring in 590 additional forage to meet feed demands, increasing production costs or reduce stocking rates. 591 Nutrient cycling: The impact of extreme weather events on soil fertility was evidenced 592 through immediate and prolonged changes in extractable soil nutrients. While soil P 593 concentrations were largely unaffected by drought stress, flooding altered soil P in different 594 directions at the various timescales. The reduction in plant-available P post flood is consistent 595 596 with the reductive dissolution of P bound to iron or manganese oxides within the clay fraction of the soil under the observed negative redox potentials (Sánchez-Rodríguez et al., 2019a; 597 Sánchez-Rodríguez et al., 2019b). The P released from the clay surfaces is prone to leaching 598 599 while the iron and manganese oxides are quickly precipitated within aerobic micro-sites leading to the accumulation of FeacOH and MnacOH. A general downward trend in soil solution 600 DRP as the flood period progressed supports this theory. This reduction in P<sub>acOH</sub> persisted for 601

the first year of recovery potentially impacting sward recovery. Hence, farmers should be 602 encouraged to test for soil P status following prolonged flooding, as this may have changed. 603 604 N dynamics in soils are tightly coupled to soil moisture (Bowles et al., 2018). Consequently, in contrast to P, soil mineral N concentrations were influenced by both 605 drought and flood stress. Suppression of plant growth combined with low soil moisture and 606 limited leaching potential during drought led to the accumulation of  $NH_4^+$  and  $NO_3^-$  in the 607 608 soil. While the accumulation of NH4<sup>+</sup> during flood stress was accompanied by no change in NO<sub>3</sub><sup>-</sup> concentration despite reduced plant N requirements. We ascribe the greater 609 610 accumulation of NH<sub>4</sub><sup>+</sup> during the flood phase compared to the drought to a combination of (1) supressed microbial nitrification at low Eh potentials during flooding, and (2) increased 611 mineralisation of organic matter introduced as dead plant material. Waterlogged conditions 612 increase the leaching potential of  $NO_3^-$ , and the rate of denitrification (Rohe et al., 2021) 613 explaining the lack of soil NO<sub>3</sub><sup>-</sup> response. The accumulation of mineral N immediately post 614 drought or flood stress provides essential nutrients for plant growth following removal of the 615 stress and is essential to the resilience of pasture systems (Oram et al., 2020). This likely 616 contributed to the lack of annual yield loss seen post drought but was not sufficient to 617 mitigate the within-event year impact of flooding on annual biomass. 618

Drought did not influence soil fertility metrics in the longer term. However, differing P<sub>acOH</sub> and NO<sub>3</sub><sup>-</sup> responses post flood and during the recovery periods suggest a longer-term impact on N and P dynamics in the flood plots. During recovery phase 2, no fertiliser was applied, and livestock were excluded, therefore the increase in  $P_{acOH}$  and  $NO_3^-$  concentrations in the flood plots suggests increased biogeochemical cycling of nutrients to replace those lost from the system. This recovery of soil fertility in the flooded plots was further evidenced by a recovery of annual biomass to that of the control plots in year 2.

The exact mechanisms for these patterns of soil fertility are unclear but may be due to 626 a change in soil-plant-microbe interactions. In the absence of fertiliser inputs, soil fertility is 627 largely driven by microbial processing of organic matter (Risch et al., 2019). Manipulation of 628 rainfall has been shown to alter soil microbial community structure (Ochoa-Hueso et al., 629 2020) and microbial activity via increased extracellular enzyme expression (Ochoa-Hueso et 630 al., 2018). The higher microbial biomass in flooded soils 1 and 2 years post stress can be 631 632 attributed to increased inputs of dead organic matter and an associated resource pulse post flooding (Wright et al., 2015). Furthermore, plant-soil feedback mechanisms are key drivers 633 634 of ecosystem processes and plant associated microbiomes are profoundly influenced by abiotic factors and changing environmental conditions (Pugnaire et al., 2019; Saijo and Loo, 635 2020). The shift in plant and microbial community composition observed during pasture 636 regrowth may have influenced the soil chemistry and the root associated microbiome, as 637 evidenced by the shift in PLFA markers, including markers for AMF, with implications for 638 nutrient acquisition strategies (Lozano et al., 2021). Further work, on the detailed changes in 639 microbial communities, especially gene expression of N and P cycling and investigation of 640 the changes in extracellular enzyme expression may further elucidate the mechanisms 641 involved. 642 Organic matter decomposition: Results from the tea-bag index found reduction in the C 643 stabilisation rate during both the flood and drought phase, and a reduction in C 644 645 decomposition rate during the flood with impacts on below ground C storage. Anoxic conditions are generally considered to decrease C decomposition and favour 646 C accumulation (Greenwood, 1961; Reichstein et al., 2013). However, while the 647 decomposition rate decreased during flooding, C storage also decreased during both the flood 648 and the drought phases. The reduction in C storage was driven by a reduction in C 649 stabilisation rates, likely due to reduced rhizodeposition. Drought has been shown to alter 650

allocation of C within plants (Bahn et al., 2013) and affect exudate quantity and quality
(Williams and de Vries, 2020) but the impact of flooding on these dynamics remains a
significant gap in the literature. Additionally, iron redox cycles during flooding can release
mineral protected C with leaching of DOC via the soil solution. Measurable changes in soil C
stores take time to develop. However, the persistent shift in microbial composition post flood,
could impact on C turnover especially if more resource-conservative stress tolerant traits are
favoured over enzyme expression (Wang and Allison, 2021).

 $\frac{Climate \ regulation:}{Climate \ regulation:} Soil plays an important role in regulating the climate and can act both as$ a sink for C and a source of GHGs (Lal et al., 2021). Flood and drought decreased CO<sub>2</sub>emissions and cumulative CO<sub>2</sub> fluxes during the stress period due to a reduction in soilmicrobial and root respiration with the largest effect on total flux seen for the combinedevents. However, the lower cumulative CO<sub>2</sub> emissions during drought in the previouslyflooded plots indicates that the negative impact of the combination of stresses on soil was notadditive.

Flooding has a significant impact on the production of CH<sub>4</sub> due to the generation of
negative redox potentials (Zhang and Furman, 2021). Supressed gas exchange under
waterlogged conditions delayed the pulse of CH<sub>4</sub> release until the removal of the flood water.
In the flooded soils, the drought period produced a switch from a CH<sub>4</sub> source to a CH<sub>4</sub> sink.
The sustained release of CH<sub>4</sub> from the flooded soils is likely due to the lag in WFPS
recovery, but changes in the microbial community structure may also play a role.

Nitrous oxide makes an important contribution to GHG emissions from grazed
pasture systems with soil moisture, N fertiliser application (Cardenas et al., 2019) and excreta
deposition (Chadwick et al., 2018) being the main drivers of N dynamics. Flood stress had
the largest impact on N<sub>2</sub>O emissions with a large pulse emitted once the floodwater was
removed and a second pulse associated with fertiliser application. During the flood and the

first days of recovery, NH4<sup>+</sup> is the predominant inorganic N form within the soil mineral and 676 solution phases as anaerobic conditions supress nitrification. Subsequent mineralization of 677 678 dead vegetation, mesofauna and soil microorganisms resulted in a pulse of C and N release facilitated by favourable summer temperatures (Kirwan and Blum, 2011; Sánchez-Rodríguez 679 et al., 2019b). This resulted in a peak in NH<sub>4</sub><sup>+</sup> during soil recovery. Concurrent nitrification 680 and denitrification occurring at microsites within the soil led to the observed N2O emissions 681 682 in the soil recovery phase after flooding (Miniotti et al., 2016; Zhu et al., 2013) and contributed to the observed decrease in  $NH_4^+$  and  $NO_3^-$ , in the soil solution. Additionally, 683 684 NH<sub>3</sub> volatilization (when the soil was flooded and saturated) would have reduced the NH<sub>4</sub><sup>+</sup> concentration in the soil solution (Verhoeven et al., 2018). During longer-term recovery, 685 increased N demand due to a resumption of the growth of plants (seed bank) and soil 686 microorganisms under non-anoxic conditions led to a gradual decline in NO<sub>3</sub><sup>-</sup> in the soil 687 solution, in line with N<sub>2</sub>O peaks. However, this reduction was slower than for NH<sub>4</sub><sup>+</sup>, which 688 indicates that the gradual recovery of plants and microorganisms which was insufficient to 689 fully utilise the available N resource. Current understanding of N dynamics in agricultural 690 systems identifies N fertilisation as the main driver of spikes in N<sub>2</sub>O emissions. However, the 691 N<sub>2</sub>O pulse following floodwater recession was greater than that produce by N fertilisation. 692 Consequently, flooding caused the highest GWP (excluding CO<sub>2</sub>) in line with Hou et al. 693 (2000), especially in combination with drought. These data can help to fill knowledge gaps 694 695 related to GHG emissions from agricultural soils (Bianchi et al., 2021). Furthermore, alteration of the soil microbial communities is likely to play a large role through the alteration 696 of N dynamics and the long-term effect of flood induced shifts in microbial structure on GHG 697 emissions requires further investigation. 698 **Pollution regulation:** The risk of erosion and runoff generation immediately post flood is 699

increased due to the severe reduction in plant cover and the high WFPS, reducing infiltration

capacity of the soil, and remains a threat for the first year of recovery along with an increased
risk of nutrient and sediment transport. Conversely, 8 weeks of drought did not increase the
proportion of bare ground present retaining soil protection to future heavy rain events,
minimising the erosion risk and impact on water quality.

Flood events have been shown to export significant amounts of P to receiving 705 waterbodies (Ockenden et al., 2017). Traditionally, P leaching has not been considered to be 706 707 a major transport pathway in clay-rich soils. However, evidence of P leaching to groundwater in certain soil, hydrologic and management conditions is growing (McDowell et al., 2019; 708 709 Stuart and Lapworth, 2016) and in flooded soils this may be enhanced by reductive dissolution cycles of Fe/Mn oxides. The general downward trend in DRP over the flood 710 period indicates loss of P to leaching while the spikes in concentration are likely due to 711 712 localised release of P from iron oxides in response to anoxic conditions developing. Further evidence of loss of P from the soil is seen through the reduction in P<sub>acOH</sub> post flood. Unlike P, 713  $NO_3^{-}$  is prone to leaching and the general downward trend in soil solution  $NO_3^{-}$ , coupled with 714 low N<sub>2</sub>O emissions during the flood phase suggest possible loss to groundwater. During this 715 period, the concentrations detected were low ( $< 0.25 \text{ mg NO}_3^- \text{L}^{-1}$ ) and unlikely to pose a 716 significant water quality risk. However, immediately post flood there is an increased risk of N 717 and P leaching due resumption of microbial activity leading to mineralisation of dead plant 718 material and nitrification processes converting accumulated  $NH_4^+$  to  $NO_3^-$ , evidenced by a 719 720 large, and environmentally significant, spike in DOC, DOP and NO<sub>3</sub>-concentrations within the soil solution followed by a sharp decline. 721

722 <u>Biodiversity provision:</u> Grasslands provide important floral resources for pollinating species.

723 However, the low biodiversity within many agricultural pastures supporting livestock

production is contributing to global declines in species (Sánchez-Bayo and Wyckhuys, 2019).

725 While drought had no impact on plant community composition, the increase in diversity and

richness recolonising the flood plots post stress may promote greater insect populations 726 including pollinating species evidenced by a persistent increase in nectar-containing species 727 728 in the flood plus drought treatment. However, in contrast grassland crop wild relatives are largely composed of flooding-intolerant grass species (Jarvis et al., 2015) and flooding 729 reduced their abundance for 1 year post-flood. Crop wild relatives contribute towards an 730 ecosystem's genetic resource provision (Jarvis et al., 2015) and often exhibit useful traits 731 732 related to stress tolerance (Castañeda-Álvarez et al., 2016). However, if flooding becomes more widespread, reduction of this valuable resource could have negative impacts for 733 734 biodiversity provision and future agricultural plant breeding programs aimed at producing plant varieties more resilient to climate change. 735

736

### 737 5. Conclusions

Extreme weather events are increasing in frequency and magnitude. Here we show that 738 flooding and drought have a significant impact on a range of above and below ground 739 indicators of ecosystem function and that this pasture system is more resistant and resilient to 740 drought than to flood. Contrary to our prediction, the compound flood+drought treatment did 741 not further exacerbate the flood impact. In fact, there was some indication of more resilience 742 in soil and microbial parameters suggesting contrasting event types may promote recovery 743 through a more rapid return to ambient soil moisture conditions. The immediate impact of 744 745 flooding on all ecosystem services was negative, especially for production and climate and water regulation. Flooding stress caused pronounced and persistent shifts in soil microbial 746 and plant communities with large implications for nutrient cycling and long-term ES 747 748 provision. However, determination of the mechanisms behind these changes and the impact of these shifts along with the complex plant-soil-microbe interactions, on whole ecosystem 749 750 function remains a key research priority. The observed increase in soil nutrient concentrations

immediately post flood and drought stress has the potential to provide a resource pulse for
pasture growth and recolonization. Soil testing is essential when determining fertiliser rates to
minimise nutrient loss and minimise impacts on water quality and GHG emissions.
Furthermore, the large pulse of N<sub>2</sub>O emissions is striking and with the projected increase in
the frequency, severity and spatial extent of flood events further research is required to factor
flood-induced N<sub>2</sub>O pulses into GHG emission projections.

757

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766

#### 767 Author contribution

Davey L. Jones, David R. Chadwick, Dylan Gwynn-Jones, Rosalind J. Dodd and Paul. W.
Hill conceived the study and set up the experiment. Rosalind J. Dodd, Antonio R. SánchezRodríguez and Simon M. Smart undertook the main fieldwork, laboratory studies and data
analysis. Rosalind J. Dodd wrote the first draft of the manuscript and all authors contributed
substantially to revisions and have given final approval of the submitted manuscript.

773

#### 774 **Conflict of interest**

The authors declare no competing interests.

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#### 1121 Figure legends

Fig. 1. Response of above- and below-ground indicators (a) immediately post flood or 1122 drought stress, (b) 1 year and (c) 2 years following the stress event. Effect sizes represent 1123 variation from the control calculated from the LnR/R response ratio as outlined in the 1124 methods. Error bars represent the 95 % confidence interval. Open symbols represent 1125 significant effects (95 % CI not overlapping Zero). The number of indicators significantly 1126 1127 different to the control at the three time-points for the three treatments are shown in (d). In all panels AMF denotes arbuscular mycorrhizal fungi. 1128 1129 Fig. 2. Ecosystem service indicator scores provided by the pasture community (a) at the 1-1130 month pasture regrowth stage post flood, (b) immediately post drought, (c) 6-months post 1131 drought, (d) 1-year post drought and (e) 2-years post drought. The letters denote significant 1132 differences between treatments at the p < 0.05 as determined by the Tukey HSD test of 1133 multiple comparisons and *n.s.* denotes no significant difference. 1134

1135

1136 Fig. 3. Mean temporal dynamics of soil solution (a) dissolved organic carbon (DOC), (b)

1137 dissolved reactive phosphorus (DRP), (c) dissolved organic phosphorus (DOP), (d)

ammonium  $(NH_4^+)$  and (e) nitrate  $(NO_3^-)$  within the flood plots during the flood phase and

1139 recovery phase 1. Error bars show the standard error of the mean.

1140

Fig. 4. Principle component analysis for PLFA (a) in the four treatments across the five
sampling dates, immediately post flood, immediately post drought and 6, 12 and 24 months
post drought and (b) vectors indicate the direction of shift towards specific taxonomic groups
85 % of the variance in PLFA markers was explained by PC1 (x-axis) and a further 10 % was

explained by PC2 (y-axis). Ellipses group the responses to the specific time period and errorsbars show the standard error of the mean for the four treatments.

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- **Fig. 5.** Greenhouse gas emissions for each treatment showing hourly CH<sub>4</sub> (a-d), CO<sub>2</sub> (d-h)
- and N<sub>2</sub>O (i-l) fluxes (mean  $\pm$  standard error, n = 4) in each period of the experiment (flood
- 1150 phase, recovery phase 1, drought phase and recovery phase 2) for the different treatments
- assessed in this study (control, flood, drought and flood + drought). The arrow and F indicate
- a fertilization event.
- 1153

- 1155 Table 1. Indicator metrics associated with the 6 selected provisioning, supporting and regulating
- 1156 services provided by agricultural grasslands.

Environment Service category	Service	Indicators	Measurement
Provisioning	Food provisioning	Biomass, forage grass cover, weed species cover	Pasture cut, plant surveys
	Biodiversity provision	Plant species richness + diversity, nectar producing species, butterfly larvae food species, crop wild relatives	Plant surveys
Regulating	Climate regulation	CO <sub>2</sub> , CH <sub>4</sub> , N <sub>2</sub> O emissions	Static chamber greenhouse gas emission measurements
	Pollution regulation	% ground cover, soil solution chemistry (DOC, NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup> , DRP, DOP) <sup>1</sup>	Plant surveys, rhizon sampling of soil solution (during flood phase only)
Supporting	Nutrient cycling	Soil extractable NO <sub>3</sub> -, NH <sub>4</sub> +, P	Soil samples
	Organic matter decomposition	C stabilisation rate (S), C decomposition rate (k)	Tea-bag index

- <sup>1</sup> DRP = dissolved reactive phosphorus, DOC = dissolved organic phosphorus, DOC = dissolved organic
   carbon.
- 1160
- 1161 Table 2. Mean cumulative GHG fluxes and total CO<sub>2</sub> equivalents with and without CO<sub>2</sub> emissions
- along with the standard error. The letters denote significant differences between treatments
- according to the Tukey's HSD test at the p < 0.05 level of significance.

Treatment	CH <sub>4</sub>	CO <sub>2</sub>	N <sub>2</sub> O	GWP (exc. CO <sub>2</sub> )	GWP (inc. CO <sub>2</sub> )
	(kg C ha⁻¹)	(kg C ha⁻¹)	(kg N ha⁻¹)	(kg CO <sub>2 eq</sub> ha <sup>-1</sup> )	(kg CO₂ <sub>eq</sub> ha⁻¹)
Control	-0.38 ±	8111 ± 192	1.01 ± 0.14 ab	273 ± 40 ab	7915 ± 274 a
	0.18 a	а			
Flood	0.98 ±	4651 ±	3.35 ± 1.03 ab	874 ± 264 ab	5238 ± 1347 a
	0.72 a	1213 ab			
Drought	-0.20 ±	4726 ±	0.41 ± 0.06 b	108 ± 15 b	4602 ± 1062 a
	0.03 a	1059 ab			
Flood &	1.49 ±	2942 ± 617	4.16 ± 1.43 a	1060 ± 343 a	3813 ± 925 a
drought	1.16 a	b			
p value	0.215	0.009	0.031	0.025	0.059

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# **E68**ure 1

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1Eigure 4

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