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Harvey, Rachel J.; Chadwick, David R.; Sánchez-Rodríguez, Antonio Rafael ; Jones, Davey L.

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Agroecosystem resilience in response to extreme winter flooding

Rachel J. Harvey[a, b], David R. Chadwick[a], Antonio Rafael Sánchez-Rodríguez[a,c,*] and Davey L. Jones[a,d]

[a] *Environment Centre Wales, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK*

[b] *Centre for Ecology and Hydrology, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK*

[c] *Agronomy Department, Universidad de Córdoba, Campus de Rabanales. Edificio C4 Celestino Mutis, 14071 Córdoba, Spain*

[d] *UWA School of Agriculture and Environment, University of Western Australia, Crawley, WA 6009, Australia*

*Corresponding author. Tel.: +34 957218915

E-mail address: antonio.sanchez@uco.es (A.R. Sánchez-Rodríguez).

Highlights

- Extreme winter flooding negatively altered soil physical, chemical and biological indicators.
- Soil available P was reduced by 42% in the flooded areas after the flood event.
- Plant biomass was reduced by 0 or 19-34% in flooded areas.
- Total soil microbial biomass was increased by 60% after flooding.
- Grassland soils were more resilient than other crops.

Abstract

Evidence suggests that climate change is increasing the frequency of extreme weather events (e.g. excessive rainfall, heat, wind). The winter of 2013-14 saw exceptional levels of rainfall across the UK leading to extreme and prolonged flooding (up to 3 months with floodwater depths up to 3 m) in several low-lying agricultural areas (e.g. Somerset Levels, Thames Valley). The impact of extreme flooding and the speed of ecosystem recovery at the field-scale, however, remain poorly understood. The main objectives of this study were therefore to: (1) assess the effect of this extreme winter flooding event on a range of soil physical, chemical and biological quality indicators at 15 flood-affected sites (arable and grassland), (2) determine if these changes in soil health were reversible in the short term (< 1 year), and (3) to evaluate the effectiveness of different mechanical interventions (sward-lifting, subsoiling, slot-seeding and aerating) to accelerate the amelioration of the damage caused by winter flooding at 2 of the 15 sites. Once the floodwater had receded (April 2014), we found that several of the measured soil quality indicators were negatively affected in the flooded areas in comparison with non-flooded areas. This included a decrease in soil bulk density (by 19%), soil pH (by 0.4 units), and available P (by up to 42%). Flooding increased soil microbial biomass (60%), induced a shift in soil microbial community structure and reduced earthworm numbers. After 8 months of recovery, only soil pH remained significantly reduced (by 0.3 units) in the flooded areas in comparison to the unflooded areas. Flooding had a negative impact on the overlying vegetation at the arable sites (biomass production was reduced by between 19 and 34%) but had no major impact at the grassland sites in the long-term. In the flood amelioration experiment, the subsoiled plots produced grass with a higher nutrient content (e.g. N - up to 35%, Ca - up to 19% and Mg - up to 58%). However, the four different interventions appeared to have little positive impact on most of the soil quality indicators measured. In conclusion, extreme winter flooding was found to induce short-term alterations in key soil quality indicators and to destroy

winter crops, although these effects did not persist in the longer term. Our results therefore indicate that the temperate agroecosystems evaluated here were highly resilient to winter flood stress and that recovery to a pre-flood state could be achieved within 1 year. Improved management strategies are still needed to speed up the rate of recovery after flood events to facilitate a faster return to agricultural production.

Keywords: Extreme weather; Nutrient cycling; PLFAs; Waterlogging.

1. Introduction

There is increasing evidence that short-term extreme weather events (e.g. excessive rainfall, heat, wind) are becoming increasing frequent globally (Donat et al., 2016), potentially leading to negative effects (i.e. floods, droughts) and threatening long-term terrestrial ecosystem functioning (Harris et al., 2018). These increases are more evident in North America and Europe in comparison with other countries located in the Southern Hemisphere (Berghuijs et al., 2017). For example, the winter of 2013-2014 saw exceptional levels of rainfall in the UK leading to extreme and prolonged flooding in many low lying areas with agricultural land remaining under water for up to 3 months (Slingo et al., 2014; Defra, 2014). Similar events have occurred in other countries such as the USA in 2011, 2013 and 2014 (Mallakpour and Villarini, 2015).

Perhaps the most obvious impact of prolonged flooding in agricultural fields is the damage to crops (Malik et al., 2002). Soil becomes anaerobic when it is waterlogged, and this has almost immediate effects on vegetation. Within 48 h, plants begin to suffer from O_2 deprivation, which causes a significant reduction in nutrient uptake rates, inhibiting plant growth both above and belowground (Jackson, 2004). If waterlogged or anaerobic conditions persist, hydrogen sulphide, acetic acid and butyric acid are produced as the soil redox potential

74 levels reduce. These compounds can be toxic to plants and can remain even after the soil has
75 dried out again (McKee and McKelvin, 1993). In more extreme cases when soils are subjected
76 to prolonged and complete submergence, the availability of CO_2, light and O_2 decrease,
77 severely reducing photosynthesis and respiration rates and ultimately leading to death in many
78 crop species (Jackson and Colmer, 2005) and a significant monetary loss to farmers
79 (Posthumus et al., 2009).

80 Soil chemistry can change considerably under waterlogged conditions leading to a
81 disruption in nutrient cycling (e.g., N, C and P) and excessive losses (Cabrera et al., 1999;
82 Sánchez-Rodríguez et al., 2017, 2018, 2019a, 2019b). Under anaerobic conditions, the N
83 mineralisation process is halted due to the lack of oxygen and as a result NH_4^+ levels build up
84 to higher than normal concentrations (Unger et al., 2009). While NH_4^+ is usually beneficial to
85 plants as a readily available form of N, in excess it can inhibit growth and even become toxic
86 to some plants (Loqué and von Wirén, 2004). Furthermore, pH can change when soils become
87 flooded (Ponnamperuma, 1972). If soil pH is altered sufficiently beyond the optimum levels
88 for plant growth, then the addition of lime or fertilisers may be necessary (Fernández and
89 Hoeft, 2009).

90 Flooding can also cause physical changes to the soil (e.g. changes in soil structure and
91 bulk density), especially in fine clay soils (Jackson, 2004). Soil aggregate stability in the upper
92 layers reduces during long-term flooding as a result of several chemical processes, particularly
93 elevated pH, increased cation exchange and the prevalence of reduced conditions
94 (Ponnamperuma, 1972). This disaggregation and compaction of surface soils decreases the
95 chance of water draining away into the subsoil and increases the chance of surface capping,
96 which can hinder plant growth and soil drying once the floodwater recedes Horn et al., 1995),
97 as well as increasing the risk of overland flow of water and pollutants.

98 Macrofaunal communities can survive short term flooding events (Zorn et al., 2005)
99 and can help alleviate some of the problems caused by flooding by burrowing to aerate the soil,
100 and transporting and releasing nutrients (Lavelle et al., 2006). However, although several
101 earthworm species can survive in aerated waterlogged conditions for some time (Zorn et al.,
102 2005), in anaerobic waterlogged conditions, macrofaunal communities can disappear due to
103 the lack of O_2 (Plum, 2005). Furthermore, soil microbial communities may change from a
104 diverse aerobic assemblage to a much less diverse and less active anaerobic community, which
105 can further contribute to changes in soil chemistry (Freeman et al., 2004).

106 To alleviate the effects of flooding on soils, the changes discussed above essentially
107 need to be reversed. Firstly, the soil needs to dry out, nutrients need to be restored and soil
108 structure needs to be improved to facilitate plant growth and further drainage and aeration of
109 the soil. On one hand, drying the soil is the crucial first step, and will remedy most of the
110 negative impacts of flooding (Ponnamperuma, 1984). On the other hand, if the soil is worked
111 by heavy machinery while it is still too wet, there is a risk that severe soil structural damage
112 can occur, especially in clay soils (Dexter and Bird, 2001). In particular, bulk density can
113 increase, water porosity decrease, aggregate stability decrease and the continuity of pores and
114 links to any drainage systems can be damaged (Dexter and Bird, 2001). To help improve
115 drainage, infiltration rates can be improved by reducing stocking density on grazed land to
116 minimise soil compaction (Castellano and Valone, 2007), planting cover crops to break up the
117 surface layers (Angers and Caron, 1998), introducing organic matter to the soil to improve soil
118 structure (Franzluebbers, 2002), or by cross field ploughing along contours rather than down
119 slopes (Puustinen et al., 2005).

120 Once the soils are sufficiently dry, heavier machinery can be used to break up the
121 compact soil (Spoor, 2006). Generally in wet soils, ploughing or sub soiling is often preferred
122 as the mechanical disturbance aerates the soil to a greater depth than other mechanical means

(generally >20 cm) (Strudley et al., 2008). Other cultivation methods include sward lifters, which aerate the soil to a depth of 20 cm, or aerators, which aerate the soil to a depth of around 10 cm (Strudley et al., 2008). However, all of these cultivation methods require a tractor to pull the equipment through the soil, which can cause compaction both on the surface and at plough depth, depending on the furrows created by each method (Spoor, 2006; Strudley et al., 2008). This can eventually result in a 'plough pan', which can then lead to further compaction and reduced drainage in the future if the soil is not dry enough (Dexter and Bird, 2001).

Due to the rarity of extreme floods, relatively little is known of the long-term impacts of prolonged inundation and subsequent recovery. Considering that we are predicted to experience more extreme flood events in the future (Slingo et al., 2014), it is imperative that we understand these impacts and, more importantly, how to mitigate and alleviate the damage they might cause. The main objectives of our study were therefore: (1) to assess the effect of the extreme UK winter flooding event (2013-2014) on physical, chemical and biological soil quality indicators at 15 flood-affected sites; (2) to determine if these changes in soil health are reversible in the short term (around 1 year), and (3) to determine the best methods for alleviating flood damage caused by extreme winter flooding at 2 of these sites (sward lifting, sub soiling, slot seeding and aeration in comparison with the control plots without intervention). Our hypotheses were: (1) if the flood water column was considerable (0.3 to 1 m), it is possible that this would have a profoundly different impact on plant production, soil biological, physical and chemical properties in comparison with a <0.3 m water column or waterlogged soils; (2) if this water remains for an extended period, as it did in winter 2013-14, perhaps even flood-tolerant crops may not be able to recover in the long term (a few months to one year).

2. Materials and methods

2.1 Study sites, experimental design, treatments and sampling timeframe

Fifteen agricultural field sites were selected across Somerset, Worcestershire, Herefordshire and North Wales to monitor the recovery of soils and vegetation after prolonged flooding (Table 1, Sites 1-15). Sites were selected to cover a number of important agricultural crops and soil types, and there needed to be clear evidence of unflooded and flooded areas at the same site. Where it was possible (Sites 1 to 7 and 13 to 15), each site was divided into 'control' areas that were those that had remained above the flood water and 'flooded' areas that were those that had remained under water for long periods of time (8-12 weeks; Fig. A1). Initial sampling took place in April 2014 (Sites 1 to 15; including floodwater samples, Table A1), just after the last of the flood water had receded, and the final samples were taken eight months later in December 2014. A subset of these sites with defined flooded and control areas (Sites 3, 4, 7, 14 and 15) were selected for a more detailed monitoring of soil recovery. Sampling was carried out on these five sites every five weeks from the end of May 2014 through to the middle of December 2014, resulting in a total of seven temporal sample points for each of these five sites. In the meantime, these sites were managed (and fertilised) as usual according to the crop grown at each one. At each site, three independent replicate plots (3 m × 3 m) were sampled from the control or flooded areas. The same replicate plots were used for sampling throughout the study. Aboveground biomass, soil respiration rate, water infiltration rate, soil bulk density, soil pH, electrical conductivity (EC) and soil nutrients (available-P, NO_3^- and NH_4^+) were determined (0-10 cm depth) at the five detailed monitoring sites for each time period. At the remaining ten sites, all the above measurements were made in April 2014 and December 2014 with the exception of soil respiration and infiltration rate, and phospholipid derived fatty acids (PLFAs) were evaluated as indicators of soil microbial biomass and community structure in April 2014 only for sites 1 to 6 and 13 to 15.

Additionally, two grassland sites in the Somerset Levels (Site 12 and 16) where the flooding was most extreme were selected for an amelioration experiment. Both of these sites had been under water for the longest period of time (12 weeks with >1 m depth of floodwater; Table 1). The experimental plots were set up 4 months after floodwater removal when the soil had dried out enough to allow heavy machinery trafficking. All treatments were slot-seeded except the control treatment and the experimental design at each site was identical and comprised four blocks ($n = 4$) of each treatment (10 m wide, 25 m long) namely: (1) unamended control, (2) sward-lifted, (3) sub-soiled, (4) aerated, and (5) slot-seeded only (called slot-seeded). The fields were sampled 4 times over a 12-month period after the experiments were initiated. The same replicate plots were used throughout the experiment. Aboveground biomass, soil respiration rate, soil infiltration rate, soil bulk density, soil pH, electrical conductivity (EC) and soil nutrients (available-P, NO_3^- and NH_4^+) were determined (0-10 cm depth) at sampling time. In addition, foliar mineral element concentrations were determined after harvesting the above-ground plant biomass from small plots (40 × 40 cm), Subsequently, the samples were dried (80°C, 72 h), ground, ashed (450°C, 24 h), the ash dissolved in HCl (Adrian, 1973) and the mineral content determined on a 700 Series ICP-OES (Agilent Technologies Inc., Santa Clara, CA).

All treatments, except the control, were slot-seeded with *Lolium perenne* L. to re-establish the pasture lost by flooding (AHDB, 2017a). The other interventions were chosen based on their ability to penetrate the soil at different depths as follows (Fig. A2):

- *Sub-soiler* (Viceroy moledrainer-subsoiler; Browns Agricultural, Leighton Buzzard, UK): the deepest treatment, penetrating to a depth of 30-36 cm. The sub-soiler consists of two tines that dig deep ruts into the soil approximately 2.5 m apart.
- *Sward lifter* (Grassland Shakaerator; McConnel Limited, Ludlow UK): the mid treatment, penetrating to a depth of 20-25 cm. The sward lifter consists of three tines

over a width of 2.5 m, preceded by a row of sharp disks to break up the surface soil and followed by a roller to flatten the turf. The sward lifter also vibrates as it is pulled through the soil.

- *Aerator* (Slitmaster Grassland Aerator; Browns Agricultural, Leighton Buzzard, UK): the shallowest treatment, penetrating to a depth of 10-15 cm. The aerator consists of several sharp points over a width of 3 m that roll over the surface of the soil creating several small holes.

These three mechanical interventions were chosen based on expert advice from local agronomists and national guidance (AHDB, 2016, 2017b).

2.2. Measurement of soil physical quality indicators

Stainless steel bulk density rings (100 cm^3; Eijkelkamp Soil and Water, Giesbeek, Netherlands) were used to take three intact cores (0-10 cm depth) from each flooded and control plot. The samples were subsequently, weighed, dried (105°C, 16 h), reweighed and dry bulk density and gravimetric moisture content calculated. Infiltration rates (ml min^{-1}) were measured in the field using a Decagon Devices mini disk infiltrometer (METER Group Inc., Pullman, WA) and calculating the average infiltration rate over a 30 min measurement period. The only exception to this was the last sampling in the amelioration trial when a single ring infiltrometer was used (Bagarello and Sgroi, 2004).

2.3. Measurement of soil chemical quality indicators

Soil samples (0-10 cm depth) from each plot were sieved to 2 mm for analyses. Deionised water (25 ml, 4 h) was used to extract 10 g of each soil sample and pH measured using a Hanna pH probe and electrical conductivity (EC) with a Jenway 4520 conductivity meter (Cole-Parmer Ltd, Stone, UK). Soil plant-available P was measured by extracting soil

with 0.5 M $NaHCO_3$ (pH 8.5; 1:5 w/v, 200 rev min^{-1}, 0.5 h; Horta and Torrent, 2007), centrifuging the extracts (14,000 *g*, 15 min) and determination of P colorimetrically in the supernatant was done according to Murphy and Riley (1952) on a Powerwave XS plate reader (BioTek Instruments Inc., Winooski, VT). Soil NH_4^+ and NO_3^- were measured by extracting 5 g of soil with 0.5 M K_2SO_4 (1:5 w/v, 200 rev min^{-1}, 1 h), centrifuging the extracts (14,000 *g*, 15 min) and colorimetric analysis of the supernatant according to Mulvaney (1996) and Miranda et al. (2001) respectively using a Powerwave XS plate reader.

2.4. Measurement of soil biological quality indicators

To determine changes in soil microbial biomass and community structure, phospholipid derived fatty acids (PLFAs) were determined on 25 g soil samples (previously sieved to 2 mm) according to Bartelt-Ryser et al. (2005) for Sites 1-6 and 13-15 (n = 4 per condition and site) immediately after the floodwater had receded (Apr. 2014). No PLFA samples were collected from sites 7-12 because the whole field was flooded and there were no suitable control areas. The soil was sieved to pass 2 mm and immediately frozen (−80°C). One-hundred twelve different fatty acids were detected in the soil samples used for PLFAs but only 32 of them had a concentration higher than 0.5 % of the total PLFAs. These thirty-two fatty acids, classified per taxonomic group, were: (1) 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, 18:0 iso, 17:0 anteiso, 15:1 iso ω9c and 17:1 iso ω9c used for Gram+ bacteria (Ratledge and Wilkinson, 1988; Kieft et al., 1994; Paul and Clark, 1996; Zelles, 1999; Olsson et al., 1999; Bartelt-Ryser et al., 2005); (2) 16:1 ω7c, 16:1 ω9c, 17:1 ω8c, 18:1 ω5c, 18:1 ω7c, 18:1 ω9c, 17:0 cyclo ω7c and 19:0 cyclo ω9c were used for Gram− bacteria (Kieft et al., 1994; Paul and Clark, 1996; Zelles, 1999); (3) 16:0 10 methyl, 17:1 ω7c 10 methyl, 18:0 10 methyl and 18:1 ω7c 10 methyl for actinomycetes (Zelles, 1999); (4) 15:0 DMA as biomarker for anaerobic bacteria; (5) 20:4 ω6c for protozoa (only 0.34 % of the total PLFAs; Paul and Clark, 1996); 18:2 ω6c for

saprotrophic fungi (Paul and Clark, 1996); (6) 16:1 ω5c as biomarker for putative arbuscular mycorrhizal fungi (Olson et al., 1999); and (7) 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0 were found but were not assigned to a specific taxonomic group (Ratledge and Wilkinson, 1988; Niklaus et al., 2003). Some PLFA ratios were calculated to assess alterations in the soil microbial communities (protozoa/bacteria or predator/prey, Gram+/Gram−, saturated/unsaturated fatty acids, mono/polyunsaturated fatty acids, and precursor/cyclo fatty acids).

Above-ground plant biomass was measured in 40 cm × 40 cm independent replicate quadrats at each site to determine differences in plant productivity between flooded and control areas. After collection, the samples were dried (80 °C, 16 h) and their dry weight determined. Earthworm numbers were quantified within a 20 × 20 × 20 cm volume of soil for each plot. The soil was excavated, hand sorted and any earthworms present counted before being returned to the plot. Soil respiration rate was measured at each plot using an EGM-4 infra-red gas analyser (PP-Systems Ltd, Hitchin, UK).

2.3. Statistical analysis

Permutational multiple analyses of variances (PERMANOVAs) were used to determine differences between conditions (flooded, control) and sites (n = 15) at the start and at the end of the observational study. The data were square root transformed, Euclidean distance dissimilarity matrices were calculated for each analysis and Partial Eta Squared effect sized (η^2_p) were calculated for PERMANOVA results, where a small effect was defined as ≥ 0.0099, a medium effect ≥ 0.0588, and a large effect ≥ 0.1379. 1-way ANOVAs were used to compare the soil and aboveground parameters between flooded and control areas both at the start and at the end of the study, including PLFAs (taxonomic groups and ratios at the start of the study only). Principal component analysis (PCA) was used for PLFAs taxonomic groups to assess

alterations in the soil microbial communities. Additional PERMANOVAs were done for each condition (flooded and control) with the factors time (start and end data) and site.

To identify seasonal changes in measured parameters at the 5 more intensively monitored sites, mixed-design ANOVAs were conducted on the monthly data to determine any significant differences between conditions (flooded and control areas) and over time (7 samplings). The same statistical analysis was used at each individual site.

The amelioration study data was analysed using PERMANOVA to determine differences between sites, treatments and over time, and for each site separately to find differences between treatments and sampling times. Additionally, 1-way ANOVAs were run for each site and the four-time samplings to find significant differences between the five treatments. An Analysis of Similarities (ANOSIM) was used to identify any significant dissimilarities between treatments at the individual sites and months. As ANOSIM is a type of regression analysis Pearson's *r* effect size was used instead of Partial Eta Squared, where a small effect is defined as ≥ 0.1, a medium effect is ≥ 0.3 and a large effect size is ≥ 0.5. Tukey´s post hoc test was done to find differences between treatments when 1-way ANOVA was significant.

When PERMANOVAs were used, pairwise tests were used to determine where any statistical differences lay (flooded vs. control areas, between sampling times and treatments) and additional PCAs were used to determine which factors explained most of the variation in the data (we only showed the principal components with a Eigenvalue higher than 1.0 and that explained more than 5% of the variance; for more details see "Appendix: Details of Statistical Analysis and Results", termed "Appendix" from now). The statistical analyses were performed using the statistical package SPSS software v22.0 (IBM Inc., Armonk, NY) and Primer-e software v6.0 (Quest Research Limited, Auckland, New Zealand).

297 **3. Results**

298 *3.1. Impact of flooding and subsequent recovery at 15 sites*

299 At the start of the observational study, there were significant differences with large
300 effect sizes between conditions (P(perm) = 0.027, η^2_p = 0.633) and sites (P(perm) = 0.001, η^2_p
301 = 0.903). A PCA analysis showed that soil moisture, soil EC and soil NO_3^- were the main
302 factors explaining 93.0% of the variance in the data (Appendix, Page 1, Table A1, three
303 principal components). On the one hand, bulk density, soil pH and soil P were significantly
304 lower in the flooded areas in comparison to the control areas (P = 0.027, P = 0.004, and P =
305 0.034, respectively; Table 2). In contrast, soil moisture and soil EC were significantly higher
306 for the flooded areas (P < 0.001 in both cases). By the end of the observational study, there
307 were no significant differences between conditions except for soil pH, where the same pattern
308 as at the first sampling was observed (P = 0.023, Table 2), although there were still significant
309 differences with large effect sizes between sites (P(perm) = 0.001, η^2_p = 0.925; Appendix,
310 Pages 1-2, and PCA in Table A2, three principal components that explained the 96.1% of the
311 variance).

312 As expected, flooded areas differed between the start and end of the study (P(perm) =
313 0.001, η^2_p = 0.621), although there were also significant differences between sites (P(perm) =
314 0.001, η^2_p = 0.881). These differences between sites were more evident when the crops were
315 different. A PCA showed that soil moisture and soil EC were the main factors explaining 87.5%
316 of the variance in the data (Appendix, Page 3, Table A3, two principal components). Similarly,
317 control areas also changed over time (P(perm) = 0.001, η^2_p = 0.783) and again showed
318 significant differences between sites (P(perm) = 0.001, η^2_p = 0.882). A PCA showed that soil
319 moisture, soil EC, soil P and soil NO_3^- were the main factors explaining 95.2% of the variance
320 in the data (Appendix, Pages 3-4, Table A4, three principal components). The fact that both

flooded and control areas differed between the start and end of the study suggests seasonal variation.

The total PLFAs and the percentage of anaerobic bacteria were significantly higher under flooded conditions than in the control areas (P = 0.018 and P < 0.001, respectively), while the opposite occurred for the percentage of fungi (P = 0.017) in April 2014 (Table 3). None of the calculated PLFA ratios were altered by flooding. The PCA showed that Gram+, Gram−, protozoa and fungi were the main factors that explained 81.5% of the variance (Fig. 1, only two principal components). After the extreme flood event (April 2014), the soil microbial communities shifted from being related to higher percentages of fungi, putative arbuscular mycorrhiza fungi and protozoa in control areas to higher percentages of Gram+ bacteria, actinomycetes and anaerobic bacteria (Sites 1, 2, 3, 4, 6, 14 and 15) or Gram− bacteria (Sites 5 and 13; Fig. 1) in the flooded areas.

3.2. Monthly monitoring of soil recovery from flooding at five sites

In general, there were significant differences over time for all the monitored variables (Appendix, Pages 4-6, Table A5 for a PCA). The main effect comparing between conditions (flooded/control areas) was significant for infiltration rates (P = 0.034, η^2_p = 0.202), soil NH_4^+ (P = 0.031, η^2_p = 0.207), soil NO_3^- (P = 0.003, η^2_p = 0.321) and plant biomass (P = 0.020, η^2_p = 0.230). However, there were significant interactions for bulk density (P = 0.005, η^2_p = 0.404), infiltration rates (P = 0.040, η^2_p = 0.328), soil EC (P = 0.039, η^2_p = 0.329) and soil NO_3^- (P = 0.004, η^2_p = 0.411).

Fig. 2 shows the time course of the soil physical properties for the five sites. The winter flood event produced an increase in the soil moisture until the end of the experiment in the flooded areas in comparison with the control areas but the differences were only significant for the sampling in September/October (P = 0.039; Fig. 2a). Bulk density (Fig. 2b) and infiltration

346 rate (Fig. 2c) were not altered by flooding but there were significant differences between
347 months for the control (August vs. September/October sampling for bulk density, $P = 0.023$;
348 July vs. August, $P = 0.019$, and September/October vs. November, $P = 0.020$, for the infiltration
349 rate) and the flooded areas (November vs. December, $P = 0.005$, for the infiltration rate). More
350 significant differences were found when looking at each site individually (Table 4). Soil
351 moisture was significantly higher in the flooded areas of the five sites for some specific months,
352 but bulk density and the infiltration rate were altered in contrasting patterns for the different
353 sites and even sampling times. Flooding reduced soil bulk density in Sites 7, 14 and 15 but it
354 was increased in Sites 3 and 4 (Table 4). Alterations in the infiltration rate of the flooded areas
355 did not follow a simple trend: for the flooded areas, it was increased at the beginning of the
356 recovery phase and later decreased in Sites 3 and 4, while it was increased at Site 14 and a non-
357 clear trend was observed at Sites 7 and 15 (Table 4).

358 Soil chemical indicators are shown in Fig. 3. Soil pH was significantly reduced in the
359 flooded areas (taking together the five sites) in July 2014 ($P = 0.031$). There was a significant
360 reduction in the soil pH between June and July for the control and the flooded areas ($P < 0.001$
361 in both cases) and an increase for the flooded areas between September/October and November
362 ($P = 0.035$; Fig. 3a). Looking at the flooded areas of each site individually, soil pH was
363 significantly higher in the flooded areas at Sites 3 and 15 (1 month for each site) and lower in
364 Sites 3, 4, 7 and 14 (1, 2, 4 and 2 months, respectively) in comparison with the control areas
365 (Table 4). A general increase was observed for soil EC of the flooded areas during the whole
366 sampling period and the five sites together, significantly for May ($P < 0.001$), June ($P < 0.013$)
367 and July ($P < 0.011$, Fig. 3b), although some decreases were observed for Sites 4 and 7 (Table
368 4). Soil EC was significantly reduced between May and June ($P < 0.030$), September/October
369 and November ($P = 0.025$) and increased between July and August ($P < 0.001$), and August
370 and September/October ($P = 0.021$, Fig. 3b).

For the five sites together, there were no significant differences for soil P, soil NH_4^+ or NO_3^- between the flooded and the control areas (Fig. 3 cde). The differences were more associated with the sampling time: there was a reduction of the soil P in the control areas in June vs. July ($P = 0.005$). A significant increase in soil NH_4^+ and NO_3^- was observed when comparing July vs. August ($P = 0.023$ and $P < 0.001$, respectively) and in soil NH_4^+ in August vs. September/October ($P = 0.050$ and $P < 0.001$, respectively) in the control areas, and in soil NH_4^+ ($P = 0.004$) in August vs. September/October and in soil NO_3^- ($P = 0.036$) when comparing July vs. August in the flooded areas. In addition, a significant reduction in soil NH_4^+ and soil NO_3^- occurred between September/October and November for the control ($P = 0.012$ and $P < 0.023$, respectively) and flooded ($P = 0.001$ and $P < 0.001$, respectively) areas. For each site (Table 4), soil P was significantly reduced in the flooded areas except in Site 15 (no significant differences), soil NH_4^+ was increased in Sites 3, 4 and 7 (two, two and one months, respectively) but decreased in Sites 14 and 15 (one and two months, respectively) in the flooded areas. Soil NO_3^- increased in Sites 3, 4, 7 and 14 (one, one, two and one month, respectively) but also reduced later in two of them, 4 and 7 (two and one months, respectively) in the flooded areas.

A clear negative effect was observed for plant biomass in May ($P = 0.004$), June ($P = 0.004$) and July ($P = 0.005$) in the flooded areas, and then, the production was significantly reduced between July and August for the control areas only ($P < 0.001$; Fig. 4a) because they were harvested. This is in line with what happened individually in Sites 3 (increased in May and quickly decreased in June), 4 and 14 but not with Site 7, where a positive effect of flooding was observed for plant production (Table 4). A negative effect was also observed in the number of earthworms and in the CO_2 flux in the flooded areas, with significant differences in August ($P < 0.001$) and November ($P < 0.001$), respectively (Fig. 4bc). There were significant differences in the number of earthworms between November and December for the flooded

areas (significant recovery of number of earthworms, $P = 0.015$) and for the CO_2 flux between August and September/October for the control ($P = 0.016$) and the flooded ($P = 0.018$) areas when we considered the five sites together. The lack of earthworms in the flooded areas of Sites 3, 4 and 15 meant that no significant differences were found between conditions individually (Table 4) in contrast with Sites 7 and 14. The effect of flooding in relation to the CO_2 was negative for Sites 3, 4, 15 and 15 but then positive for Site 7 (Table 4).

3.3 Mechanical interventions to promote amelioration of the soil after extreme flooding

An overall analysis of both trial sites was conducted to find any overarching patterns, however, there were no significant effects of treatment on soil indicators, although there were significant differences between months (P(perm) = 0.001, $\eta^2_p = 0.750$) and sites (P(perm) = 0.001, $\eta^2_p = 0.833$; Appendix, Page 7). A PCA showed that soil EC and soil P were the main factors explaining 96.2% of the variation in the data (Appendix, Page 7, Table A6, two principal components).

Looking at each site individually, Site 12 showed significant differences between treatments (P(perm) = 0.001, $\eta^2_p = 0.166$) and months (P(perm) = 0.001, $\eta^2_p = 0.850$; Appendix, Pages 7-8). A PCA showed that soil EC was the main factor explaining 93.9% of the variation in the data (Appendix, Page 8, Table A7, one principal component). Then, Site 16 showed significant differences between treatments (P(perm) = 0.022, $\eta^2_p = 0.127$) and months (P(perm) = 0.001, $\eta^2_p = 0.828$). A PCA showed that soil EC and soil P were the main factors explaining 97.0% of the variance in the data (Appendix, Page 9, Table A8, two main components).

Focusing on each site and time of sampling separately, a small number of significant differences were found, although these contrasted between sites (Figs. 5, 6 and 7). Soil bulk density was decreased when the aerator and the slot seeder ~~only~~ were used for Site 12 in August 2015 ($P = 0.027$), while for Site 16 bulk denisty increased in the order slot seeded ≥ aerated =

Commented [DC1]: I am not sure what this means?

subsoiled = sward lifted ≥ control treatment in October 2014 (P = 0.025) and slot seeder ≥ aerated ≥ subsoiled = control treatment ≥ sward lifted in August 2015 (P = 0.025; Fig. 5a). Although no differences in infiltration rate were found for the different treatments, a large increase was observed on the last sampling occasion (August 2015) in comparison with the three first ones (Fig. 5b).

Soil pH was significantly reduced for the different treatments in relation with the control plots (significantly only for aerated and slot seeded plots) in December 2014 (P = 0.003) and February 2015 (P < 0.001) for Site 12, while the opposite occurred for Site 16 in three of the four samplings (P = 0.004 in October 2014, P = 0.050 in February 2015, and P = 0.002 in August 2015; Fig. 6a). The rest of the chemical indicators were significantly altered by the different treatments just once for each of them (Figs. 6 bcd). Soil P and NH_4^+ concentrations were reduced in the slot seeder plots in August 2015 for Site 12 only (P = 0.016) and in the aerated plots in December 2014 for Site 16 (P = 0.050), respectively, in comparison with the control plots (Fig. 6c). In December 2014, significantly higher concentrations of soil NO_3^- were measured in the slot seeded plots than in the sward lifted and subsoiled plots for Site 12 (P = 0.016), and in the control plots than in the sward lifted plots for Site 16 (P = 0.036, Fig. 6d).

Not many significant differences were found in the biological soil properties (Fig. 7). Significant differences between treatments were found only in February 2015 for the above-ground plant biomass in the order slot seeded ≥ sward lifted = control treatment = aerated ≥ subsoiled for Site 12 (P = 0.043, Fig. 5a). In the case of the CO_2 flux, we observed significant differences in October 2014, with the control treatment plots emitting more CO_2 than the aerated plots and then the rest of treatments (P = 0.002), and August 2015, when the control plots were the ones emitting the minimum amount of CO_2 and the aerated plots the maximum, for Site 16 (P = 0.011; Fig. 7c). Finally, some nutrient concentrations in the aboveground

biomass on each site were significantly higher in the grass grown on the subsoiled plots for Sites 12 (N and Mg) and 16 (Ca) than in the grass grown on the control plots (Table 5). Additional information is shown in the Appendix (Pages 10-11, Tables A9, A10)

4. Discussion

4.1. Soil recovery assessment

It is well established that the damage to crops and loss of soil quality under flooding is dependent on various factors including: soil and crop type, duration of event (Jackson, 2004; Jackson and Colmer, 2005), type of flooding (Sánchez-Rodríguez et al., 2018, 2019b), the agricultural practices in the flooded area before the event (Sánchez-Rodríguez et al., 2017), and the time when the event occurred (winter/spring/summer/autumn; Sánchez-Rodríguez et al., 2019a). Some of these factors, such as crop type and agricultural practices related to them, partly explains the variability in agroecosystem response observed between our sites (see also Figs. A3, A4, A5, A6, A7, A8, A9, A10, A11). Our results also indicate how difficult is to predict the effects of a prolonged flooding event on soil physical, chemical and biological indicators. Here, we highlighted the importance of repeatedly monitoring a wide range of soil quality indicators which may alter quickly over time (e.g. soil moisture, bulk density, pH, EC). Despite this, it was difficult to identify consistent trends across the sites.

4.1.1. Flood-induced changes in soil physical indicators

Flooding may cause alterations in soil structure and induce compaction (Jackson, 2004). Contrary to expectation, however, soil bulk density was actually lower in the flooded areas of the fifteen sites assessed in April 2014 and at three of the five sites evaluated monthly in comparison with the non-flooded areas (decrease of 19%), however, this was only apparent for Site 15 at the end of the monitoring period (December 2014). The lack of loss of soil

structure is consistent with no effect on soil water infiltration rate (Horton et al., 1994), as bulk density was altered it is still possible that structure was affected by flooding. As we did not directly measure structure or aggregate stability, further studies are required to critically evaluate how they respond to flooding. The use of machinery to sow, fertilize, and aerate the soil too quickly after floodwater removal (i.e. too wet) may also have contributed to more isolated physical damage at some sites, for example in Sites 3 (spring onions, Fig. A4), 4 (swedes, Fig. A5) and 13 (grassland, Fig. A10) where soil erosion, more exposure of the roots and a loss of soil structure were observed. In contrast to our study, severe degradation of soil structure has been described in sites where the crop was either sown or harvested in autumn and in newly established grasslands (Holman et al., 2003). Probably the most severe impact of flooding occurs when the floodwater moves across the field in which case a complete loss of topsoil can occur (Fig. A4, Fig. A10).

4.1.2. Flood-induced changes in soil chemical indicators

A good example of how difficult was to identify consistent trends across the sites was pH, a key soil indicator that affects nutrient bioavailability and soil microbial communities. After the floodwater had receded (April, 2014), the pH was significantly lower across the 15 test sites (Table 2, 0.4 units lower) and again in the monthly sampling (July 2014, 5 soils; Fig. 3a), but was increased for Sites 3 and 15 in one of the samplings (June and Nov. 2014, respectively). The increase for acid soils such as Site 15 can be explained by the reduction of Fe or Mn under anaerobic conditions and the pH decrease for the more alkaline soils due to increased partial pressure of CO_2 (due to the lack of O_2) that promotes the production of H^+ (for example Sites 3, 4, 7 and 14; Ponnamperuma, 1972). The rise in soil moisture observed in the flooded areas after the flood event in the flooded areas was expected (94% higher in comparison with the non-flooded areas), as was the increase in EC (104% higher) due to the

496 release of soluble salts from decaying vegetation and lack of plant demand. However, these
497 parameters are highly dependent on topography (soluble salts can be transported to places in
498 the landscape that are prone to being flooded) and whether the floodwater originated from
499 groundwater rise or overland flow.

500 Changes in soil conditions from aerobic to anaerobic under flooding and then back to
501 aerobic conditions, not only affects soil pH, but also nutrient dynamics and their bioavailability
502 (Figuereido et al., 2015). During flooding, adsorbed and occluded P may have been released
503 from the surfaces of Fe (Figs. A9, A11) and Mn minerals as they become progressively reduced
504 by the microbial community (Delgado and Torrent, 2000). In addition, P may be released from
505 senescing vegetation (Sánchez-Rodríguez et al., 2019b). While this P may be susceptible to
506 leaching, depending on the direction of water flow in the soil profile, it could also be re-sorbed
507 onto Al hydroxide surfaces or precipitated (Schärer et al., 2009). The initial decrease in P
508 bioavailability observed across our fifteen sites is consistent with a loss of P from the plant-
509 available pool (up to a 42% in comparison with the non-flooded areas) suggesting that extra P
510 fertiliser may be required to promote optimal crop growth.

511 In relation to available N in soil, no clear pattern emerged across the sites. The
512 significant increase in soil NH_4^+ measured in the flooded areas of Sites 3, 4 and 7 could be a
513 result of continued mineralisation of organic matter during the flood period combined with the
514 inhibition of nitrification due to the lack of O_2 (Unger et al., 2009). In addition, part of this soil
515 NH_4^+ and NO_3^- could have been immobilized by soil microorganisms or taken up by plants as
516 they started growing after floodwater removal. The transformation of NH_4^+ into NO_3^- by
517 nitrifiers, whose activity was inhibited during the flooding and partially during the soil recovery
518 (high soil moisture; Nielsen, 1996), could explain the increases in soil NO_3^- in the flooded
519 areas of Sites 3, 4, 7 and 14. Some sites received fertilizers during the soil recovery phase to

improve soil fertility for the next agricultural season, explaining the increase in soil EC and P at the end of the monitoring period.

4.1.3. Flood-induced changes in soil biological indicators and plant growth

Plant biomass was negatively affected in the first few months after flooding, being between 66 to 81% lower than in the control areas. Although Posthumus et al. (2009) and Sánchez-Rodríguez et al. (2019a) showed how damaging summer floods can be on primary production, our study exemplifies the destructive effect of a prolonged winter flooding, especially when the crops are submerged for long periods. Nevertheless, this study also highlights the importance of plant species. Overall, flooding decimated the spring onion, swede and winter wheat crops while having no major effect on the grassland.

Our results showing a flooding-induced decline in earthworm populations are in general agreement with Ivask et al. (2012). In that study, it was concluded that the loss of earthworms under prolonged flooding indicated a loss of soil functionality. While we agree with this in the short-term, our results strongly indicate that earthworm numbers recover within 1 year to those seen in the unflooded controls. This implies that a loss of soil function is transitory if flood events occur very infrequently (Coyle et al., 2017; Posthumus et al., 2009).

Soil respiration rates as well as microbial activity are good indicators of soil health, but they are highly responsive to temperature and soil moisture and thus highly seasonal (Pendall et al., 2004). Although we observed changes in microbial community structure and biomass, this appeared to have little effect on soil respiration, indicating a high degree of functional redundancy within the soil community. Despite this, the microbial biomass was 60% higher after the floodwater had disappeared from the flooded areas in comparison with the unflooded areas. We ascribe this microbial growth to the increased availability of labile carbon and nutrients from the plant and microbial necromass formed during flooding. The increase in the

545 percentage of anaerobic bacteria and the reduction in fungal biomass (-28.4%) in comparison
546 with the non-flooded areas (mainly obligate aerobes) have been described previously under
547 prolonged flooding in a range of ecosystems (Freeman et al., 2004; Sánchez-Rodríguez et al.,
548 2017). Of note, is the loss of arbuscular mycorrhizal fungi which may have a long-term
549 negative impact on plant performance (particularly in low input systems) as well as potentially
550 affecting the crop's ability to withstand further stress events (Latef et al., 2016).
551
552 *4.2. Strategies to improve soil quality after prolonged flooding*
553 Overall, we observed few positive soil and sward responses to the four mechanical
554 interventions at our two trial sites. This was surprising given that these approaches are being
555 recommended to farmers to improve soil health in flood-affected areas (AHDB, 2016, 2017a).
556 In part, these recommendations are based on the assumption that flooding induces a loss of soil
557 structure and induces compaction, although this view is not supported by our multi-site study
558 (Fig. 2b). At both trial sites, soil bulk density was already low and no restrictions to root growth
559 are expected (i.e. >1.4 g cm^{-3}). However, we did observe that the dead mat of vegetation and
560 thin layer of silt (ca. 3 mm deep) on the soil surface did appear to inhibit grass emergence and
561 prolonged anaerobic conditions at the soil surface, at least in the short-term (Fig. A4). The
562 aerator and slot seeding would have helped to break this surface layer. At Site 12, all four
563 treatments proved successful at lowering bulk density although this was best in the slot-seeding
564 only treatment which received minimal vehicle trafficking. At Site 16, however, the opposite
565 effect was observed. Based on visual inspection, we ascribe the increase in bulk density to
566 compaction induced by vehicle trafficking (e.g. compression along tyre tracks) clearly
567 illustrating that the response is site-specific.
568 Tillage operations to enhance soil aeration have been shown previously to reduce
569 earthworm density (Lees et al., 2016). Although earthworm numbers in the soil were very low

after flooding, there rate of recovery was not positively influenced by any of the interventions. This is probably linked to the lack of observable response in many of the other soil quality indicators and no increase in plant productivity, both of which are strongly liked to earthworm abundance (Blakemore, 1997). In terms of plant growth, slot-seeding into the damaged sward failed to promote greater biomass production, even though the plants visibly established. This reflects our observations at other sites and from laboratory studies that older swards (Sites 12 and 16) are more resistant to winter flooding than newly established swards and can regenerate relatively quickly (Sánchez-Rodríguez et al., 2019b).

Our results showed a different response to the four mechanical interventions at the two sites. This is consistent with previous studies showing highly variable agronomic responses, with both increases and decreases in soil quality and grass productivity reported (Bhogal et al., 2011). These studies have suggested that mechanical soil loosening can be effective in improving soil structure and increasing grass yields where soil compaction has been positively identified and mechanical alleviation is effectively carried out. Where no compaction is identified (as in our trials), it appears that while soil loosening improves soil physical properties, it may reduce grass yield due to sward and root damage (Frost, 1988). Consequently, we conclude that a pre-assessment of soil quality is undertaken before any remedial work is undertaken after an extreme flooding, rather than relying on broad scale agronomic guidance notes. Further work is also required to evaluate whether our treatments would have caused a more positive impact if they had been applied at arable sites where soil structure and compaction is typically greater.

5. Conclusions

Our field-based study clearly shows that extreme winter flooding can alter a range of soil physical, chemical and biological indicators which may impact on the ability of soils to

595 deliver a range of ecosystem services. Primary productivity was heavily impacted in the winter-
596 sown arable cropping systems studied here, resulting in all cases to a loss of harvestable product
597 (between 0 and 19-34%). In contrast, much less of an effect of flooding was seen in the
598 grasslands, presumably as these perennials were better established and possess physiological
599 traits that make them more flood tolerant. Our data therefore lends support to the reduction in
600 arable cropping within high flood risk areas and a move towards land uses with greater soil
601 coverage (i.e. less erosion prone), more water storage capacity and which contain flood-tolerant
602 plants (e.g. grasslands, wetlands; Wang et al., 2012; Kharel et al., 2016). Our data also suggest
603 that more work is required to promote land restoration after extreme floods. The four
604 mechanical interventions trialled here showed little overall agronomic impact, however, these
605 options were based solely on government and industry guidance rather than on soil testing. In
606 some cases, basic soil testing would have proved beneficial to identify which soil properties
607 were sub-optimal, of which some can be easily rectified (e.g. pH) but others less so (e.g.
608 earthworms).

609 More studies like this are needed to better understand the different effects of extreme
610 flood events on agricultural production and soil quality with soil as a provider of ecosystem
611 services. It is difficult to predict extreme weather events and consequently studies such as ours
612 lack both in-field replication and field measurements prior to the event (i.e. preventing a robust
613 before-after-control-impact (BACI) design; Conner et al., 2016). Further, we lack
614 measurements of soil quality during the flood event itself. We therefore encourage more
615 replicated field experiments that can simulate prolonged flood events. In addition, it would be
616 useful to combine this with other common extreme events such as drought or ozone stress
617 which may occur at different times of the year (i.e. does flooding increase the severity of the
618 next stress event, or does it help build agroecosystem resilience?). It would also be beneficial

to gain a wider assessment of extreme flooding on soil functioning, including nutrient cycling, the persistence of pests and diseases, greenhouse gas emissions and alterations in subsoils.

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Figure captions

Fig. 1 Changes in soil microbial community structure after an extreme flood event at 9 agricultural sites. Principal component analysis for the different taxonomic groups (based on PLFAs) as a function of the sites (n = 9) and conditions (flooded and control areas) immediately after floodwater removal (April, 2014). Principal component 1 vs. 2 (a), principal component 1 vs. 3, and the corresponding taxonomic groups for these subfigures (b and d). Symbols represent the mean of four replicates per site and condition.

Fig. 2 Temporal changes in soil physical properties after exposure to an extreme flood event. Fifteen agricultural sites were monitored after the floodwater receded in April 2014. Values represent means ± SE (n = 15) for paired flooded and unflooded areas. The presence of asterisk/s indicate significant differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$) between conditions.

Fig. 3 Temporal changes in soil chemical properties after exposure to an extreme flood event. Fifteen agricultural sites were monitored after the floodwater receded in April 2014. Values represent means ± SE (n = 15) for paired flooded and unflooded areas. The presence of asterisk/s indicate significant differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$) between conditions.

Fig. 4 Temporal changes in soil biological properties after exposure to an extreme flood event. Fifteen agricultural sites were monitored after the floodwater receded in April 2014. Values

840 represent means ± SE (n = 15) for paired flooded and unflooded areas. The presence of
841 asterisk/s indicate significant differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$) between
842 conditions.
843
844 **Fig. 5** Effect of four different amelioration treatments (sward lifting, aeration, subsoiling and
845 slot-seeding) on soil physical properties at two grassland sites heavily impacted by an extreme
846 flood event. Time course (mean value and standard error; n = 4 per treatment) of soil physical
847 properties for the different treatments. The presence of different letters indicates significant
848 differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$) between treatments.
849
850 **Fig. 6** Effect of four different amelioration treatments (sward lifting, aeration, subsoiling and
851 slot-seeding) on soil chemical properties at two grassland sites heavily impacted by an extreme
852 flood event. Time course (mean value and standard error; n = 4 per treatment) of soil physical
853 properties for the different treatments. The presence of different letters indicates significant
854 differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$) between treatments.
855
856 **Fig. 7** Effect of four different amelioration treatments (sward lifting, aeration, subsoiling and
857 slot-seeding) on soil biological properties at two grassland sites heavily impacted by an extreme
858 flood event. Time course (mean value and standard error; n = 4 per treatment) of soil physical
859 properties for the different treatments. The presence of different letters indicates significant
860 differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$) between treatments.