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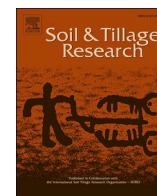
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# Soil quality regeneration by grass-clover leys in arable rotations compared to permanent grassland: Effects on wheat yield and resilience to drought and flooding

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## ABSTRACT

Intensive arable cropping depletes soil organic carbon and earthworms, leading to loss of macropores, and impaired hydrological functioning, constraining crop yields and exacerbating impacts of droughts and floods that are increasing with climate change. Grass and legume mixes traditionally grown in arable rotations (leys), are widely considered to regenerate soil functions, but there is surprisingly limited evidence of their effects on soil properties, resilience to rainfall extremes, and crop yields. Using topsoil monoliths taken from four intensively cropped arable fields, 19 month-old grass-clover ley strips in these fields, and from 3 adjacent permanent grasslands, effects on soil properties, and wheat yield in response to four-weeks of flood, drought, or ambient rain, during the stem elongation period were evaluated. Compared to arable soil, leys increased earthworm numbers, infiltration rates, macropore flow and saturated hydraulic conductivity, and reduced compaction (bulk density) resulting in improved wheat yields by 42–95 % under flood and ambient conditions. The leys showed incomplete recovery compared to permanent grassland soil, with modest gains in soil organic carbon, total nitrogen, water-holding capacity, and grain yield under drought, that were not significantly different ( $P > 0.05$ ) to the arable controls. Overall, grass-clover leys regenerate earthworm populations and reverse structural degradation of intensively cultivated arable soil, facilitating adoption of no-tillage cropping to break out of the cycle of tillage-driven soil degradation. The substantial improvements in hydrological functioning by leys will help to deliver reduced flood and water pollution risks, potentially justifying payments for these ecosystem services, especially as over longer periods, leys increase soil carbon sequestration.

## 1. Introduction

Achieving sustainable soil management to meet increasing human demands from ecosystem services including food, fibre and fuel, carbon sequestration, clean water, biodiversity and flood mitigation (Brevik et al., 2018) presents a formidable challenge that is increasingly

exacerbated by climate change (IPCC, 2019). Prior to the widespread use of chemical fertilizers, herbicides and pesticides in the 20th Century, soil fertility and crop health in Europe and North America were maintained by rotations that included 2–3 year leys comprising mixtures of grasses and nitrogen fixing legumes such as clovers (Ball et al., 2005; Knox et al., 2011; Persson Bergkvist and Kätterer 2008). Intensification

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of production has led to continuous annual cropping and short rotations focused on the most profitable crops, for example in the US Midwest “corn belt” just two crops- maize and soybean cover 70 % of the arable land area (National Agricultural Statistics Service (NASS), 2016). In much of Europe, North Africa and Asia, farmers have similarly specialised in growing cereals in short rotations, with a small number of annual break crops, but have experienced plateauing or falling yields associated with soil degradation, especially declines in soil organic carbon (SOC) (Ball et al., 2005; Squire et al., 2015; Ghosh et al., 2019; Yigezu et al., 2019).

Depletion of SOC is a global problem that reduces soil fertility, impairs hydrological functions and degrades structure, and contributes to anthropogenic greenhouse gas emissions, together making it harder to achieve the key UN sustainable development goals: 1. No Poverty, 2. Zero Hunger, 6. Clean Water, 12. Responsible Consumption and Production, 13. Climate Action, 14. Life Below Water, and 15. Life on Land (Keesstra et al., 2016). Loss of SOC is the single most important contributor (47 %) to the estimated £1.2 billion per year costs of soil degradation in England and Wales (Graves et al., 2015). The second most important contributor (39 %) to these economic losses is soil compaction, which normally increases with declining organic matter content, and the third (12 %) is erosion, which increases with compaction. Soil bulk density (BD), a measure of compaction, was found to increase exponentially with declining SOC in the UK Countryside Survey, with densities above  $1.5 \text{ g cm}^{-3}$  only found in soils containing less than 3.1 % SOC by weight (Emmett et al., 2010). Arable soils are highly vulnerable to compaction as, for example in England, average SOC concentrations have fallen to 2.5 %, with about 50 % of silt and clay soils reported 15 years ago, to hold less than 1.3 % SOC (King et al., 2005). They are likely to have declined further due to ongoing continual annual cropping in short rotations (Knight et al., 2012) and widespread use of intensive cultivation (Townsend et al., 2016).

Decreasing SOC is a direct consequence of intensification of production of cereals and other annual crops that give low returns of organic matter to soil. For example, grain comprises over 50 % of the above-ground biomass of winter wheat (AHDB, 2018a), and about 34 % of wheat straw is sold from UK arable farms (Townsend et al., 2018), including to four purpose-built power stations that together can burn over a million Mg per year (Spackman, 2017). Wheat has short-lived roots that cease growth by early June (AHDB, 2018a) typically contributing only  $0.4 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  to soil (Sun et al., 2018), whereas perennial grass-clover ley roots can add over  $1.0 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  (McNally et al., 2015). Root C decays more slowly than shoot-C and so disproportionately contributes to SOC (Sokol et al., 2018). Mixed farming has declined with intensification and stockless arable production is now widespread, whereas formerly leys were grazed or provided hay and silage off-take with manure returned, often with straw (Townsend et al., 2018), and the manure helps to maintain earthworm populations (Tiwari, 1993), and SOC content (Maillard and Angers, 2014).

Strong linear relationships occur between carbon inputs, aggregate stability, and SOC (Kong et al., 2005). Intensive cultivation of annual crops using mouldboard ploughing and harrowing, as has been typical in the UK (Townsend et al., 2016), and accounts for 28 % of cultivated area in US in 2017 (Zulauf and Brown, 2017), degrades these soil components by increasing mineralization of SOC. Furthermore, intensive cultivation depletes earthworm (Crittenden et al., 2014) and mycorrhizal fungal populations (Lu et al., 2018) that are important agents in soil aggregation and carbon stabilization (Tisdall and Oades, 1982; Wilson et al., 2009; Zhang et al., 2013), and depletion of these organisms reduces the protection of SOC. Declining SOC has been seen in longitudinal studies spanning from 1940–2007 during which time there has been agricultural intensification (King et al., 2005; Emmett et al., 2010; Kirk and Bellamy, 2010). In the latter study, carbon dynamics in soil under continuous arable ( $n = 525$  sites), arable rotations with leys ( $n = 302$  sites), and permanent pastures ( $n = 601$  sites) was modelled from UK national soil data from 1978–2003. Compared to continuous cropping,

soils with leys in their rotation had 12 % greater C inputs, 30 % lower C turnover rates and 40 % greater C stocks, whereas the permanent grasslands had similar inputs to arable soils, but 33 % lower turnover rates, resulting in 50 % greater C stocks (Kirk and Bellamy, 2010).

Consistent with these findings, grasslands maintain better soil quality and support a wider range of ecosystem services than arable fields, including greater SOC, and improved infiltration and water-storage capacity associated with reduced BD (Holden et al., 2019). Grassland soils are both more resistant (able to function during imposed stress) and resilient (able to recover functioning following a period of stress) to biological and physical stresses than arable soils, these responses being positively correlated with SOC content (Gregory et al., 2009). Reintroduction of leys into arable rotations might be expected to improve the resistance and resilience of arable soils to increasing severity and frequency of both drought and heavy rainfall events associated with climate change (Lowe, 2018; Samaniego et al., 2018), but this does not appear to have been investigated to date. However, there is increasing recognition that leys can help to improve soil functioning and wheat crop yields (Persson et al., 2008; Prade et al., 2017; AHDB, 2018b).

The main focus of research on effects of leys on soils has concerned their role in maintaining or increasing SOC stocks in arable rotations over many decades (Kirk and Bellamy, 2010; Poeplau and Don, 2015; Johnston et al., 2017; Prade et al., 2017). Since rates of SOC gain by leys are small relative to existing stocks (Johnston et al., 2017), where leys have been reintroduced into permanent arable land for a few years the evidence of their benefits to SOC stocks are more ambiguous. New 1–2 year grass-only leys in five arable fields showed a trend for a 5 % increase in SOC in the top 10 cm of the soil but this was not statistically significant (Gosling et al., 2017). Similarly, introducing a 2 year grass-clover ley in a 6 year arable rotation in Switzerland had no effect on SOC from 0–20 cm depth, but it increased large macroaggregates  $>2 \text{ mm}$  by 65 % (Puerta et al., 2018), emphasising the need to study the responses of a broader range of soil quality indicators, especially in early stages of arable-to ley conversions. With the increasing frequency of extreme weather events linked to climate change (Blöschl et al., 2019) it is important to establish if leys increase the resilience of soils and crop production to these stresses, and could help reduce risks of flooding impacting agriculture and property. Evidence of effectiveness of land management practices for improving soil quality and hydrological functions (organic matter, infiltration rates, hydraulic conductivity, porosity, bulk density, and water holding capacity) degraded by intensive arable cropping has recently been reviewed by Chapman et al. (2018). They concluded that the majority of studies have focused upon use of less intensive tillage methods and that critical knowledge is lacking with regard to the ability of other options, including leys, to improve most of these key components of soil quality and functioning.

To address these knowledge-gaps, we investigated the effects of reintroduction of grass-clover leys in arable rotations after decades of tillage and annual cropping mainly with cereals, as is now common in many parts of the world, on soil physical, chemical and biological properties, hydrological functions and crop performance. Our aim was to determine the extent to which short-term (19 month old) grass-clover leys introduced into arable fields improve topsoil hydrological functioning, earthworm populations and wheat yields, including crop resilience to simulated 4-week long drought and flood events during stem elongation in May. These treatments simulated the kinds of weather-stress events that are increasing in frequency within the UK, and more widely in temperate regions, in response to climate change (Lowe, 2018). Continuous arable and permanent grassland soils gave starting point and endpoint benchmarks against which the extent of differences in soil quality and crop resilience in the leys were evaluated. Our central hypothesis was that short-term grass-clover leys introduced into intensively cultivated arable fields help to restore the performance and resilience of soil functions towards those of permanent grasslands.

## 2. Materials and methods

### 2.1. Study site soils

The experiment used soil taken from four arable fields and three pastures at The University of Leeds farm, a commercial mixed farm in northern England, UK (53° 52' 25.2 N 1° 19' 47.0" W), see Supporting Information Fig. S1. The soil is a loamy, calcareous brown earth 50–90 cm deep, underlain by dolomitic limestone, and in the Aberford series of Calcaric Endoleptic Cambisols which covers an estimated 1125 km<sup>2</sup> of arable land in England and Wales (Cranfield University, 2019).

### 2.2. Arable, ley and pasture treatments

Three of the four arable fields were ploughed, harrowed and cropped with conventional management since 1995 and one field was in permanent pasture from 1998 to 2008, and then returned to the same continuous annual cultivation and cropping as the other three fields (see Supporting information Table S1). In all the arable fields wheat was grown in about 60 % of the rotation, with oilseed rape, barley, potatoes and vining peas as break crops. The chemical inputs to the arable fields during the ley establishment are detailed in Holden et al. (2019). Two of the pasture fields had been in permanent grassland since 1998, and one had been converted to arable from 2002 to 2011 but was re-sown to ryegrass in 2012 (see supporting Table S1).

In April 2015 pairs of strips 3 m wide and 70 m long, perpendicular to one edge of each arable field and approximately 40 m apart, were established by spraying the winter wheat crops with glyphosate. In May 2015, the strips were cultivated using a triple till subsoiler with shallow tines and pressing wheels, and sown in late May to early June with a grass-clover seed mixture. This comprised two varieties of tetraploid *Lolium x boucheanum* (12 % and 16 %), diploid and tetraploid *Lolium perenne* (20 %, and 16 %, respectively), *Festulolium* spp., 16 %, *Trifolium repens* 5 %, and *Trifolium pratense* 15 %, at an application rate of 4.2 g m<sup>-2</sup>. The area of the arable fields between the ley strips received the same management as the rest of the field, and served as controls.

### 2.3. Monolith excavation from the fields

In November 2016, 45 intact blocks of soil hereafter referred to as monoliths, were excavated from the fields to fit into plastic boxes 37 cm long, 27 cm wide, and 22 cm deep. The boxes had nine 10 mm drainage holes drilled into their bases, which were lined with 0.5 mm pore size nylon mesh to prevent loss of soil or earthworms through the holes. Triplicate monoliths were removed, at 68 m from the field edge, from the centre of each of the ley strips, from the control arable area between them, and from the pasture fields. Velcro strips were attached to the inner edge of the top of monolith boxes as it is effective in restricting earthworm movements (Lubbers and van Groenigen, 2013; Andriuzzi et al., 2015).

All of the arable fields had been sown with winter barley (*Hordeum vulgare*) in autumn 2016. At the stage of monolith removal these seedlings were at early stages of tillering and less than 10 cm tall, so were easily removed with minimal soil disturbance. The leys (19 months old at this point), and pasture monoliths were treated in mid-December with 0.11 g diquat as dibromide in 55 mL water, to kill the vegetation, and once senesced, the shoots were clipped and removed.

### 2.4. Monolith incubation outdoors with ambient, drought and flood treatments

The monoliths were transported in January 2017 to the University of Sheffield Arthur Willis Environment Centre, Sheffield UK (N 53°22'51" W 1° 29'58") which has a mean annual precipitation of 801 mm and mean annual temperature of 9.5 °C (Cropper and Cropper, 2016). Winter wheat seedlings of variety 'Skyfall', that had been sown in

October 2016 in an arable field adjacent to the fields with ley strips at Leeds University farm, were carefully removed from the field and root systems were washed free of soil, and transplanted from 23<sup>rd</sup>–31<sup>st</sup> January at field density (30 seedlings per monolith). The seedlings were inserted in two rows in 30 cm long slots cut in the soil to 5 cm depth, simulating direct drilling soil disturbance.

The three monoliths per field location were each assigned to a separate outdoor bench, and were placed in two rows on 100 mm thermal insulation board which was also attached around the outside of the grouped monoliths. Ammonium nitrate fertilizer was applied in April and early May at a rate of 0.48 g per monolith to give 50 kg N per ha<sup>-1</sup>. This supports about 50 % of maximum wheat yield (Hawkesworth, 2014), thereby ensuring any fertility-building by the legume-rich ley was not masked.

Once wheat stem elongation had started, for a period of 28 days from 4<sup>th</sup> May 2017, an open-sided transparent rain shelter was installed to manipulate water supply to give control and two weather-stress treatments. These were (1) ambient- average monthly rainfall for May (42.6 mm) applied in three equal weekly applications, (2) drought- no water, (3) flood- monolith box drainage holes were sealed with bungs and tap water was added until the soil was submerged with ~ 3 cm standing water and maintained at this level. On the 30<sup>th</sup> May the rain shelter was removed, drought and ambient monoliths were watered to field capacity by the addition of 2 L water each, and bungs removed from the flooded soil monoliths. From then on, all monoliths received ambient conditions until the end of the study (October 2017) when the soil was removed for analysis and to count earthworms.

### 2.5. Monolith soil moisture content

From 1<sup>st</sup> February 2017 monolith weights were recorded fortnightly over the growing season using a hanging scales weighing to 20 g precision. When the monoliths were harvested they were first weighed before soil cores of approximately 400 g were removed and their fresh and oven-dry (105 °C) weights determined. The moisture content of these subsamples were used to retrospectively calculate dry weights and fortnightly water contents of the entire monoliths.

The water-holding capacity of the monoliths was determined from their weight in late February, when the soil was at field capacity due to typical winter rain, using a retrospective calculation from the fresh and dry weight values taken later in the year at harvest, as just described above.

### 2.6. Wheat crop performance

Wheat shoots were harvested on the 11<sup>th</sup> September 2017 by cutting at the soil surface, were oven dried at 80 °C to a constant mass, and total shoot, ear, and grain weights were recorded. Grain filling was determined by counting the number of grains for each monolith.

### 2.7. Bulk density of surface soil and whole monoliths

Following the wheat harvest, surface soil (2–7 cm depth) BD was measured using a 5 cm diameter corer (Eijkelpamp, Holland), with the soil oven dried at 105 °C for 48 h and weighed. The BD of each monolith was calculated based on their volumes and total fresh weight at harvest, converted to dry weight by determining the moisture content of the approximately 400 g subsample, and also taking into account the total weight and volume of large stones > 2 cm in each monolith, extracted during sampling earthworms (see 2.10).

### 2.8. Soil hydrological properties

After wheat harvest, crop stubble was removed and a thin layer of moist silica sand was applied to the soil surface, following Holden et al. (2019), to ensure optimal contact between a mini disk tension



infiltrometer (Decagon Devices, Inc) and the soil. Infiltration rates through three classes of pore size were measured at -0.5 cm, -3 cm and -6 cm tensions which excludes flow through pore sizes of diameter > 6 mm, > 1 mm and > 0.5 mm respectively. Infiltrometry measurements were taken at the soil surface and at a depth of 10 cm. Infiltration rates were calculated from steady state flow rates as described by Reynolds and Elrick (1991). Saturated hydraulic conductivity ( $K_s$ ) was measured from each 2-7 cm depth BD core collected from the monoliths (prior to oven drying) using an Eijkelkamp 25 place laboratory permeameter.

## 2.9. Surface soil carbon and nitrogen concentrations

The 2–7 cm depth BD cores, (see 2.7), were dried and weighed and sieved (2 mm) to remove stones before being re-dried (105 °C) and weighed, and then powdered in an agate ball-mill (Fristch Pulverisette). Inorganic C was removed by adding 500 µL of 6 M HCl to 90 mg of each soil sample, and leaving for 24 h after mixing. The acid supernatant was pipetted off and the remaining soil placed in an oven (105 °C) to evaporate off any remaining acid. Duplicate 35–40 mg samples of both acid-treated and untreated soil were analysed using an Elementar Vario EL cube.

## 2.10. Earthworm populations and stone removal

After harvesting the wheat, monolith soil was sieved through a 1 cm riddle and earthworms were collected and separated into adults (those with a visible clitellum) and juveniles. The number of individuals and the fresh biomass of adults and juvenile earthworms were recorded per monolith, but will include intestinal soil, which has been reported to range from 5 to 11 % of earthworm fresh weight, depending on the species (Dalby et al., 1996). Stones retained on the riddle were washed, oven dried (105 °C), and weighed.

## 2.11. Statistical analysis

All analysis was performed in R studio version 3.5.1 (The R Foundation for Statistical Computing, 2018). Data were analysed using 2-way ANOVA with 'land use' (ley, arable or pasture) and 'weather stress' (ambient/flood/drought) as the two factors. Tukey HSD was used as a posthoc test to calculate significant differences between treatments ( $P < 0.05$ ). Data that showed variance proportional to the means were log or log +1 transformed to meet the ANOVA test requirements, including the  $K_{sat}$  and infiltration rate data sets. To test for differences in soil moisture content between field-management treatments over the fortnightly measurements across the growing season and their interaction with time, a two-way repeated measures ANOVA was conducted separately for monoliths exposed to drought, flood and ambient conditions.

## 3. Results

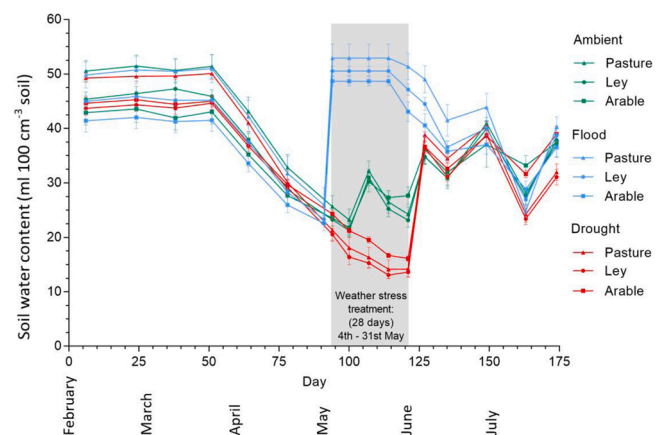
### 3.1. Monolith water content in response to ambient and weather-stress treatments

The amount of water stored in the monoliths from the start of February (Day 1) to mid-March was fairly constant, and likely reflected the field capacity of the soil from each land use (Fig. 1; for separate plots by weather-stress treatments see Supporting Information Fig. S3). Soil moisture content varied between land use during this period ( $P < 0.001$ ), which was significantly greater in the pasture soils ( $50.39 \pm 0.47$  mL water 100 cm<sup>-3</sup>) compared to arable soils ( $43.09 \pm 0.52$  mL 100 cm<sup>-3</sup>). The ley soils were intermediate, holding on average ( $45.22 \pm 0.39$  mL water 100 cm<sup>-3</sup>), which was significantly more than the arable soils but less than that of pasture ( $P < 0.05$ ).

The rainfall in April 2017 (15.5 mm) was substantially below the long-term average (1955–2019 of 59.4 mm) (Sheffield Weather, 2019) and the soil moisture decreased progressively in all of the monoliths

falling to 17–32 mL 100 cm<sup>-3</sup> by the 4th May when the weather-stress treatments commenced (Fig. 1). In the drought treatment the water content continued to fall, with the lowest values seen in the ley and pasture soils (ley  $13.6 \pm 0.8$  mL 100 cm<sup>-3</sup>, pasture  $14.2 \pm 1.5$  mL 100 cm<sup>-3</sup>). The arable soils supported wheat plants that were smaller, and presumably transpired less, as they maintained a soil water content of  $16.1 \pm 0.5$  mL 100 cm<sup>-3</sup>. The ambient treatment in which water was added to simulate long-term average rainfall in May experienced a partial recovery in soil water content. The flooding treatment, which includes the weight of water above the soil surface, showed consistently higher water storage capacity in the pasture ( $52.9 \pm 2.6$  mL 100 cm<sup>-3</sup>) than arable soil ( $48.7 \pm 0.9$  mL 100 cm<sup>-3</sup>) with the ley intermediate ( $50.6 \pm 0.9$  mL 100 cm<sup>-3</sup>). The pasture values on flooding were only slightly higher than their "field capacity" values between February and March.

On rewetting the soil in the ambient and drought treatments, the field capacity of the soils appeared to have decreased compared to earlier in the year, and their water content then oscillated until the harvest in response to evapotranspiration and ambient rainfall (103 mm in June, 72 mm in July and 79 mm in August). Drainage of the flooded soil resulted in a gradual drying and convergence of water contents with those of the former drought and ambient treatments (Fig. 1).



**Fig. 1.** Mean ( $\pm 1$  standard error) soil water content for monoliths subjected to ambient, drought and flood treatments, from 1<sup>st</sup> February (Day 1) to 25<sup>th</sup> July 2017. Replication per treatment: pasture ( $n = 3$ ), arable ( $n = 4$ ), ley ( $n = 8$ ). For plots separated by ambient, flood and drought treatments see Supporting Information Fig. S3.

### 3.2. Wheat grain yield responses to land use, weather-stress, and their interactions

Grain yield was strongly affected by land use ( $P < 0.001$ ) and weather-stress ( $P < 0.001$ ) and their interaction ( $P = 0.002$ ) (Fig. 3a; Table 1). Under ambient conditions, pasture and ley gave significantly greater yields (by 160 % and 95 % respectively) compared to the arable soils (Tukey test,  $P < 0.05$ ). Similarly, under flood conditions pasture and ley gave 76 % and 42 % higher yields than the arable soils, but only the difference between pasture and arable was significant (Tukey test,  $P < 0.05$ ). The drought treatment reduced grain yields especially on pasture and ley, to converge with the low yields of arable soils, so there were no differences between land use treatments (Tukey test,  $P > 0.05$ ).

Differences in grain yields were a result of effects both on grain filling, and numbers of grains (Fig. 3b,c). Grain filling was significantly impacted by land use ( $P = 0.04$ ) weather-stress ( $P < 0.001$ ) and their interaction ( $P < 0.001$ ) (Fig. 3b; Table 1). Under ambient conditions, grain filling was significantly lower on arable compared to ley and pasture soils (Tukey test,  $P < 0.05$  in both cases). Under both flood and drought conditions there were no significant differences in grain filling

**Table 1**

Summary of two-way ANOVA (general linear model) results for testing the effects of land use (arable/ley/pasture) and weather stress (ambient/flood/drought) treatments upon wheat crop performance at harvest, and earthworm numbers and biomass. Significant results ( $P \leq 0.05$ ) are indicated in bold; non-significant results ( $P > 0.05$ ) are denoted 'ns'.

Biological response variables	Land use <i>P</i>	Weather stress <i>P</i>	Land use x Weather stress <i>P</i>
<b>Wheat performance</b>			
Grain biomass (g)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.002</b>
Chaff biomass (g)	<b>&lt;0.001</b>	ns	ns
Shoot biomass (no ears) (g)	<b>0.005</b>	ns	ns
Total above ground biomass (g)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	ns
Mean individual grain biomass (g)	<b>0.041</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Total grain number	<b>&lt;0.001</b>	<b>&lt;0.001</b>	ns
Shoot heights at harvest (cm)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	ns
<b>Earthworm populations</b>			
Earthworm numbers	<b>0.001</b>	ns	ns
Earthworm fresh biomass (g)	<b>0.004</b>	ns	ns

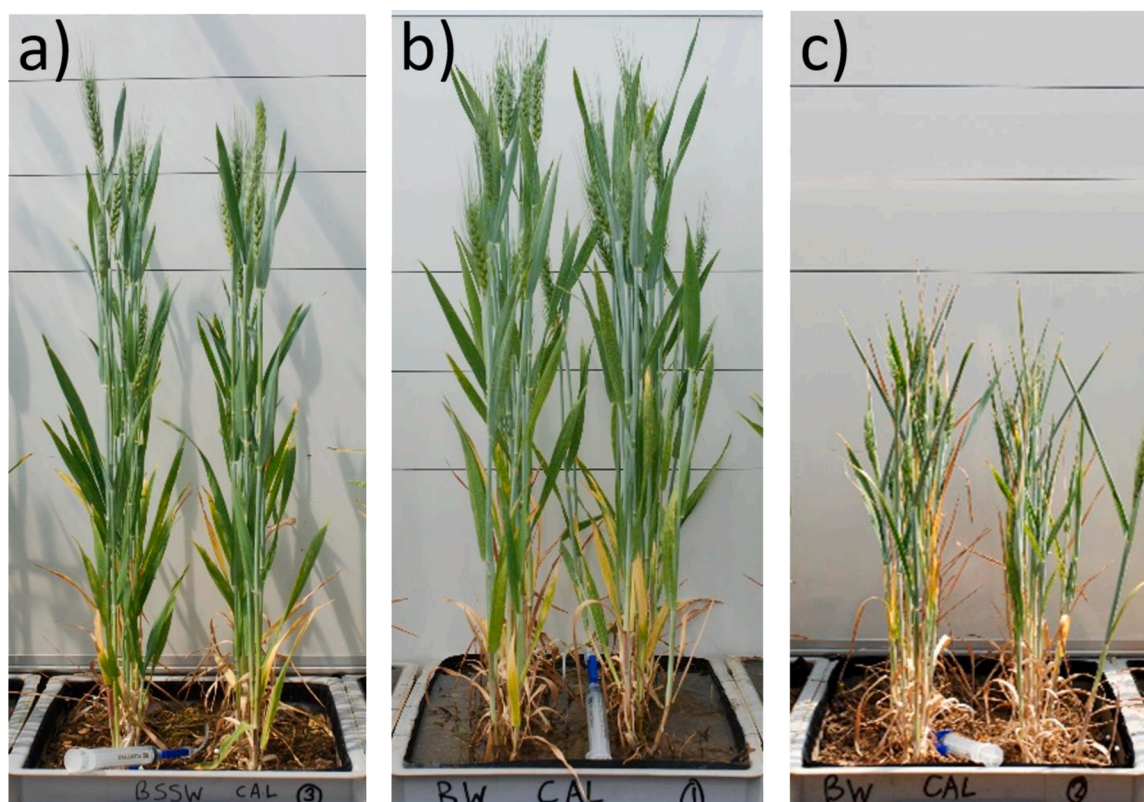
between the land use treatments, but drought significantly reduced grain filling on pasture and ley (Tukey test,  $P < 0.05$ ) compared to flood or ambient treatments (Fig. 3b).

Grain number was affected by both land use ( $P < 0.001$ ) and weather-stress ( $P < 0.001$ ) but not their interaction (Fig. 3c; Table 1). Under ambient and flood conditions pasture soils produced more grains than arable soils (Tukey test,  $P < 0.05$ ) while ley soils were intermediate and not significantly different to the other land uses (Tukey test,  $P > 0.05$ ). Grain numbers were lowest under drought, and although pasture and ley soils tended to produce more grains, they did not differ significantly to those on arable soil (Tukey test  $P > 0.05$ ).

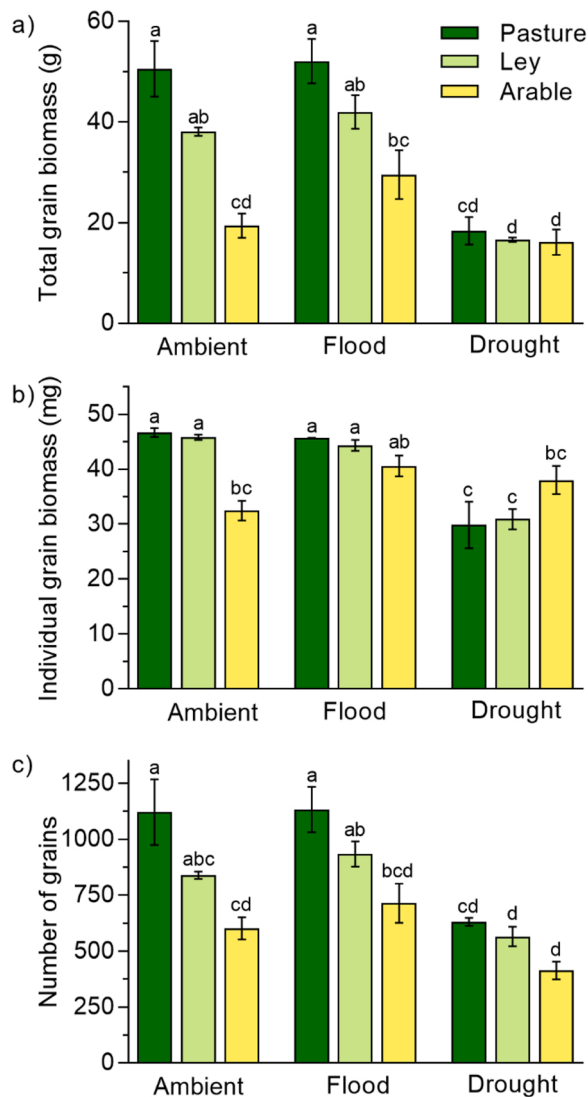
### 3.3. Overall effects of land use on wheat shoot biomass

Although the wheat plants had shown large differences in height, leaf area and shoot colour at the end of the weather stress period at the end of

May (Fig. 2; Supporting Information Figs. S4 and S5), the final shoot biomass, excluding the ears, in August 2017 showed no significant differences between drought, flood or ambient conditions (Table 1). However, there were significant overall effects of land use on grain, (Fig. 4a), chaff (Fig. 4b), and total shoot biomass (Fig. 4d) with pasture supporting significantly more biomass than the leys and the leys significantly more than the arable (Tukey tests,  $P < 0.05$ ). Excluding the grain weight, the remaining shoot biomass (Fig. 4c) showed a similar pattern with pastures supporting 82 % more biomass than the arable soils (Tukey test,  $P < 0.05$ ), and the leys showing 46 % greater biomass than the arable soils, but in this case the leys were not significantly different to either arable or grassland management (Tukey test,  $P > 0.05$ ).



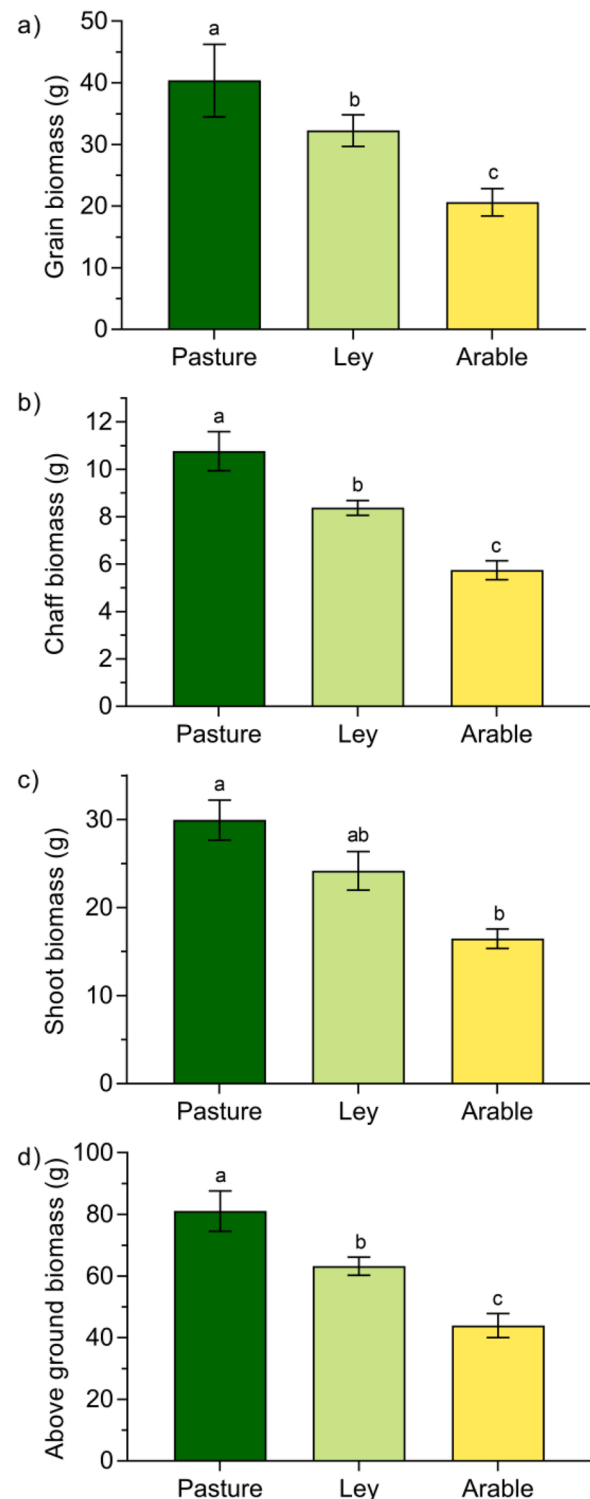
**Fig. 2.** Typical wheat growth on ley soil at the end of the weather-stress period under (a) ambient, (b) flooding and (c) drought conditions. Horizontal scale lines on the background are 10 cm apart vertically. For analysis of shoot height responses to land use and weather-stress see Supporting Information Figs. S4 and S5.



**Fig. 3.** Effects of land use and weather-stress on wheat (a) grain yield; (b), grain filling; and (c) grain numbers. Where bars share the same letter code they are not significantly different in Tukey post-hoc tests ( $P > 0.05$ ). Error bars show  $\pm 1$  SE with replication pasture ( $n = 3$ ), ley ( $n = 8$ ), arable ( $n = 4$ ).

### 3.4. Earthworm population responses to weather-stress and land use treatments

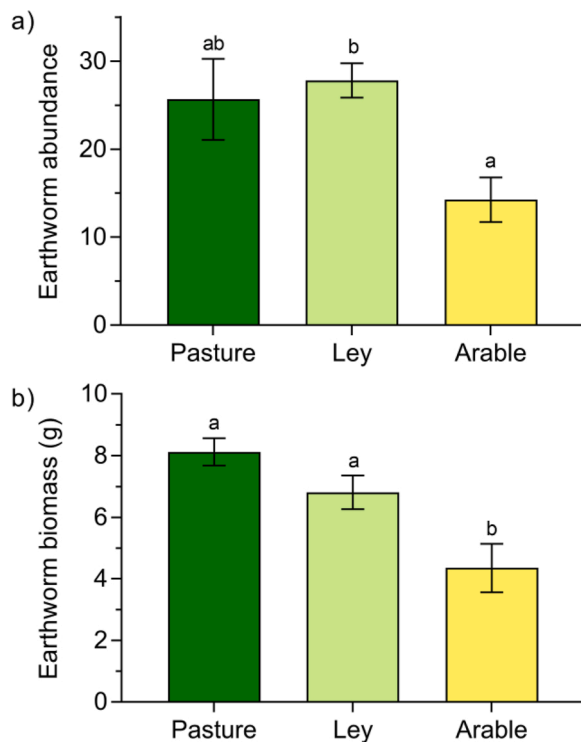
The flood and drought treatments in May 2017 did not significantly affect earthworm numbers or biomass in October (Table 1). However earthworm numbers ( $P = 0.001$ ) and total biomass ( $P = 0.004$ ) were significantly affected by land use (Fig. 5) with the leys supporting about double the numbers in the arable monoliths (Tukey test,  $P < 0.05$ ), and slightly (but not significantly) exceeding the numbers in pasture monoliths. Earthworm biomass in both pasture and ley monoliths was significantly greater than in arable monoliths. Expressed as means  $m^{-2}$  of soil and their standard errors, earthworm numbers ranged from  $146.1 \pm 26.0$  individuals  $m^{-2}$  in the arable soil, to  $285.0 \pm 20.0$  individuals  $m^{-2}$  in the former ley, and  $263.2 \pm 47.1$  individuals  $m^{-2}$  in the pasture. Earthworm biomass ranged from  $44.6 \pm 8.1$  g  $m^{-2}$  in the arable, to  $69.9 \pm 5.6$  g  $m^{-2}$  in the former ley, and  $81.7 \pm 4.3$  g  $m^{-2}$  in the pasture. Proportions of adults and juveniles were similar between soil treatments: arable (78 % juveniles), ley (77 % juveniles) and pasture (72 % juveniles).



**Fig. 4.** Overall effects of land use on wheat biomass (g per monolith) in (a) grain, (b) chaff, (c) shoots (excluding ear), and (d) total above ground biomass. Where bars share the same letter code they are not significantly different in Tukey post-hoc tests ( $P > 0.05$ ). Replication: pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ), error bars show  $\pm 1$  SE.

### 3.5. Soil organic carbon responses to land use

SOC concentrations at 2–7 cm depth in the monoliths in October 2017 (Fig. 6a) were strongly affected by land use ( $P < 0.001$ ; Table 2), being significantly higher in the pasture soil (3.07 %,  $\pm 0.24$ ) than both



**Fig. 5.** Mean number and fresh weight of juvenile and adult earthworms within soil monoliths after harvest (October 2017). Where bars share the same letter code they are not significantly different in Tukey post-hoc tests ( $P > 0.05$ ). Replication; pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ), and error bars show  $\pm 1$  SE.

ley ( $1.62\% \pm 0.05$ ) and arable ( $1.51\% \pm 0.05$ ) soil (Tukey test,  $P < 0.05$ ). The SOC of the pasture soils are typical for UK permanent pastures ( $3\% \text{ C} \pm 0.08$ ; (Fornara et al., 2011)), while the concentrations in the arable soils were within the typical range of fine textured arable soils (King et al., 2005). The total soil nitrogen (Fig. 6b) followed a similar pattern to SOC, and also showed highly significant land use effects ( $P <$

$0.001$ ; Table 2); with pasture soil ( $0.343\% \pm 0.028$ ) containing more N than both ley ( $0.171\% \pm 0.005$ ) and arable ( $0.168\% \pm 0.004$ ) soils (Tukey test,  $P < 0.05$ ). A highly significant ( $P < 0.001$ ) positive linear regression between SOC and total nitrogen content (Fig. 6c) was shown for all samples ( $R^2 = 0.96$ ), giving a constant C:N ratio across treatments ( $9.26 \pm 0.12$  SE,  $n = 45$ ). No effect of weather-stress or interaction between land management and weather-stress was found for SOC, N or C:N ratio (Table 2).

### 3.6. Soil bulk density responses to land use

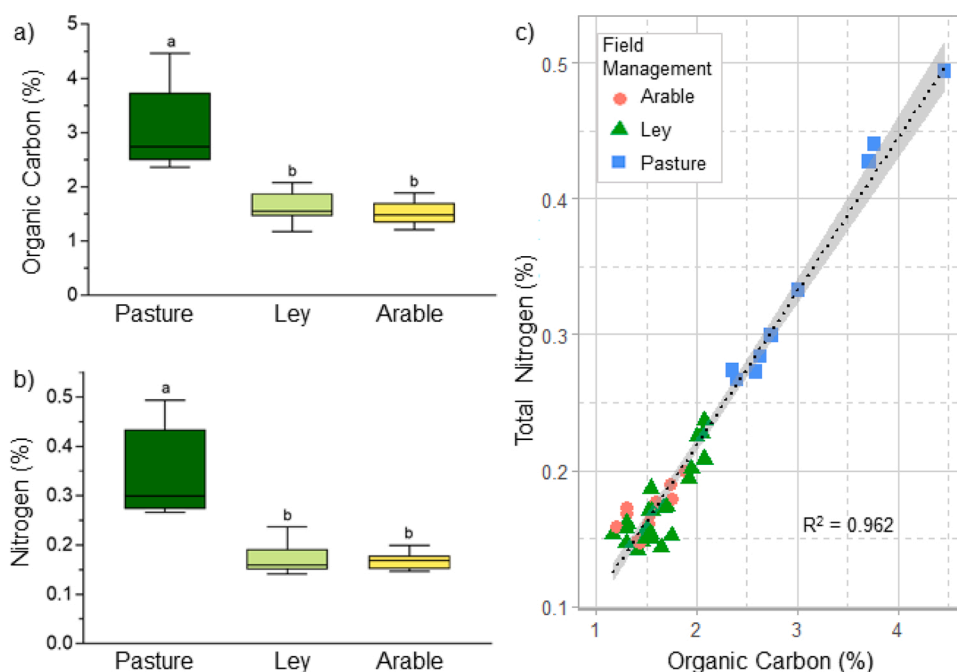
Whole monolith BD (Fig. 7a) after wheat harvest showed the pasture soils to be significantly less compacted than the arable soils ( $\text{BD } 1.24 \pm 0.04 \text{ g cm}^{-3}$  vs  $1.48 \pm 0.03 \text{ g cm}^{-3}$ ; Tukey test,  $P < 0.05$ ). The ley BD was intermediate between these two treatments ( $1.37 \pm 0.03 \text{ g cm}^{-3}$ ) and not significantly different to them (Tukey test,  $P > 0.05$ ). The BD of the upper topsoil at 2–7 cm depth (Fig. 7b) showed significant differences ( $P < 0.001$ , Table 2) between all three land uses (Tukey test  $P < 0.05$ ), with lowest values again in the pasture ( $1.17 \pm 0.04 \text{ g cm}^{-3}$ ), followed by ley ( $1.38 \pm 0.01 \text{ g cm}^{-3}$ ) and then the permanent arable ( $1.49 \pm 0.03 \text{ g cm}^{-3}$ ).

### 3.7. Soil hydrological properties in response to land use

We only report the effects of the land use treatments as no effect of weather-stress or interaction between weather-stress and land use treatments were found (Table 2) for any of the hydrological properties measured (water-holding capacity, infiltration rates, proportion of flow through different pore sizes, or saturated hydraulic conductivity).

Water holding capacity of pasture soils ( $49.8 \pm 0.9 \text{ mL } 100 \text{ cm}^{-3}$  soil) was significantly greater than that of both ley and arable soils ( $P < 0.001$ ; Table 2) which held  $44.6 (\pm 0.6)$  and  $42.9 (\pm 1.0) \text{ mL } 100 \text{ cm}^{-3}$  soil respectively (Fig. 8a). This equated to a 16 % higher water-holding capacity in pasture and a (non-significant) 4 % increase in the ley compared to the arable soil.

Saturated hydraulic conductivity ( $K_s$ ) was significantly affected by land use ( $P = 0.038$ , Fig. 8b). The back-transformed (antilog) mean  $K_s$  values of the pastures ( $49.6 \text{ mm h}^{-1}$ ) were over eight times higher than those of arable soils ( $5.84 \text{ mm h}^{-1}$ ). That of the leys ( $36.0 \text{ mm h}^{-1}$ ) was



**Fig. 6.** The concentration (% dry weight) of (a) total soil organic carbon, (b) total soil nitrogen, and (c) the relationship between them ( $\text{N}(\%) \text{ soil dry weight} = -0.00827 + 0.113 \text{ organic C}(\%) \text{ dry weight}$ ;  $R^2 = 0.962$ ;  $P < 0.001$ ; square symbols are pasture, triangles ley and circles arable) for soils collected at 2–7 cm depth after wheat harvest in October 2017. The box plots show median, interquartile range, maximum and minimum. Where bars share the same letter code they are not significantly different in Tukey post-hoc tests ( $P > 0.05$ ). Replication per treatment: pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ).



**Table 2**

Summary of two-way ANOVA (general linear model) results for testing the effects of land use (arable/pasture/ley) and weather stress (ambient/flood/drought) treatments and their interactions upon monolith soil properties. Significant results ( $P \leq 0.05$ ) are indicated in bold; non-significant results ( $P > 0.05$ ) are denoted ns. \*Log+1 transformed.

Soil chemical, structural and hydrological variables	Land use <i>P</i>	Weather stress <i>P</i>	Land use x weather stress <i>P</i>
<b>Soil chemistry</b>			
Soil organic carbon	<b>&lt;0.001</b>	ns	ns
Soil total nitrogen	<b>&lt;0.001</b>	ns	ns
C:N ratio	ns	ns	ns
<b>Soil structure</b>			
Bulk density (0–7 cm) ( $\text{g cm}^{-3}$ )	<b>&lt;0.001</b>	ns	ns
Bulk density monolith ( $\text{g cm}^{-3}$ )	<b>0.010</b>	ns	ns
<b>Soil hydrological functions</b>			
Water holding capacity	<b>&lt;0.001</b>	ns	ns
Saturated hydraulic conductivity $K_s$ ( $\text{mm h}^{-1}$ )*	<b>0.038</b>	ns	ns
Surface infiltration rate $\text{mm h}^{-1}$ (< 6 mm pores)	<b>&lt;0.001</b>	ns	ns
Surface infiltration rate $\text{mm h}^{-1}$ (< 1 mm pores)	ns	ns	ns
Surface infiltration rate $\text{mm h}^{-1}$ (< 0.5 mm pores)	ns	ns	ns
10 cm depth infiltration rate $\text{mm h}^{-1}$ (< 6 mm pores)	ns	ns	ns
10 cm depth infiltration rate $\text{mm h}^{-1}$ (< 1 mm pores)	ns	ns	ns
10 cm depth infiltration rate $\text{mm h}^{-1}$ (< 0.5 mm pores)	ns	ns	ns
Surface: proportion flow (1–6 mm pores)	ns	ns	ns
Surface: proportion flow (0.5–1 mm pores)	ns	ns	ns
Surface: proportion flow (< 0.5 pores)	<b>&lt;0.001</b>	<b>0.03</b>	ns
10 cm depth: proportion flow (1–6 mm pores)	ns	ns	ns
10 cm depth: proportion flow (0.5–1 mm pores)	ns	ns	ns
10 cm depth: proportion flow (< 0.5 mm pores)	ns	ns	ns

over six times higher than for the arable soils, but not significantly different to them or the pasture (Tukey test,  $P > 0.05$  on log transformed data, Fig. 8b).

At the soil surface, overall infiltration including flow through all pores < 6 mm was affected by land use ( $P < 0.001$ ) (Fig. 9; Table 2) with higher flow rates reported for pasture ( $752.4 \pm 64.8 \text{ mm h}^{-1}$ ) compared to arable soils ( $291.6 \pm 20.2 \text{ mm h}^{-1}$ ). The leys were intermediate between the pasture and the arable soils, with significantly higher flow rates for pores < 6 mm ( $453.6 \pm 36.0 \text{ mm h}^{-1}$ ) compared to the arable soils (Fig. 9; Table 2).

Both pasture and ley soils showed a reduced proportion of flow through < 0.5 mm pores compared to arable soils (pasture 2.5 %; ley 4.9 %; arable 8.8 %) ( $P < 0.001$ ; Fig. 10; Table 2). However, no significant differences were shown between land use treatments for the proportion of flow through 0.5 mm–1 mm or > 1–6 mm pore sizes at the soil surface (Table 2). At 10 cm depth, land use had no effect upon infiltration rate or proportion flow through any of the pore size classes measured (Table 2), so these data are not shown.

### 3.8. Relationships between soil quality indicators, and wheat yields

Significant but weak positive linear regressions were shown between SOC and water holding capacity ( $R^2 = 0.284$ ,  $P < 0.001$ ), while significant negative regressions were found between SOC and bulk density ( $R^2 = 0.675$ ,  $P < 0.001$ ), and between soil bulk density and water holding capacity ( $R^2 = 0.309$ ,  $P < 0.001$ ), (Fig. 11a–c). Positive linear regressions were shown between SOC and grain yield for the ambient ( $R^2 = 0.514$ ,  $P = 0.002$ ) and more weakly for flood stress treatments ( $R^2 = 0.269$ ,  $P = 0.047$ ) but not in the drought treated plants ( $R^2 = 0.021$ ,  $P > 0.05$ ), (Fig. 12a–c). Similarly, grain yield increased in relation to soil water holding capacity for ambient ( $R^2 = 0.345$ ,  $P = 0.02$ ) and flood treated plants ( $R^2 = 0.639$ ,  $P < 0.001$ ) but not drought conditions ( $R^2 = 0.105$ ,  $P > 0.05$ ) (Fig. 12d–f). Grain yield decreased linearly with increasing bulk density under ambient ( $R^2 = 0.733$ ,  $P < 0.001$ ) and flood stress treatments ( $R^2 = 0.471$ ,  $P = 0.004$ ), again there being no relationship in the drought treatment ( $R^2 = 0.003$ ,  $P > 0.05$ ) (Fig. 12g–i). Similar, but weaker linear regressions were found between these soil variables and total shoot biomass (Supporting information Fig. S6).

## 4. Discussion

### 4.1. Improvement of arable soil quality and functions by leys relative to permanent grassland

The introduction of grass-clover leys for 19 months into typical

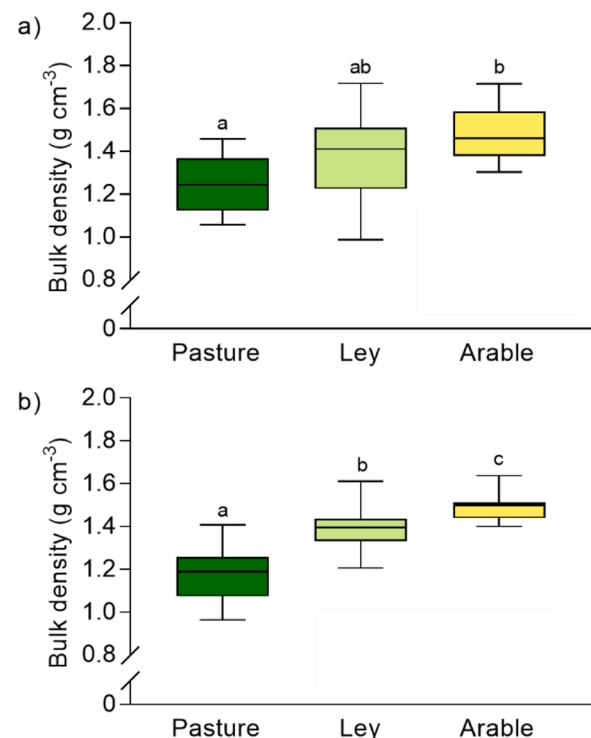
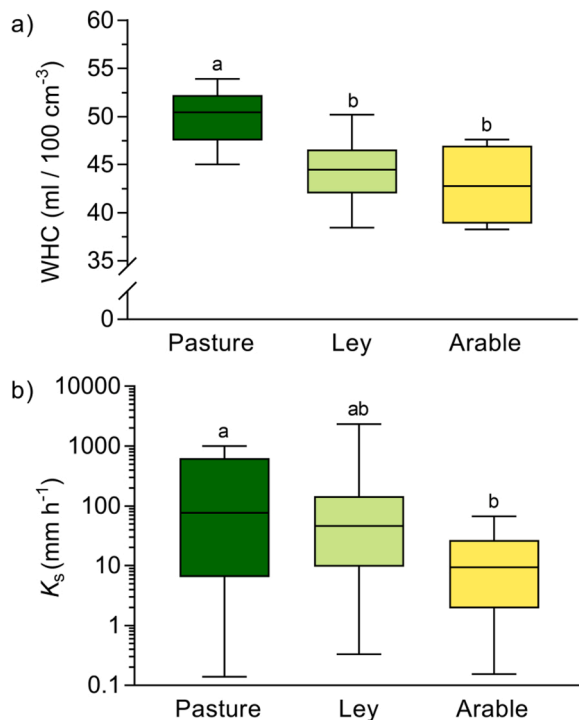


Fig. 7. Soil bulk density in October 2017 for (a) entire monoliths to their full depth of 22 cm, (b) surface soil 2–7 cm depth. Box plots show median, inter-quartile range, maximum and minimum. Where bars share the same letter code they are not significantly different in Tukey post-hoc tests ( $P > 0.05$ ). Replication per treatment: pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ).



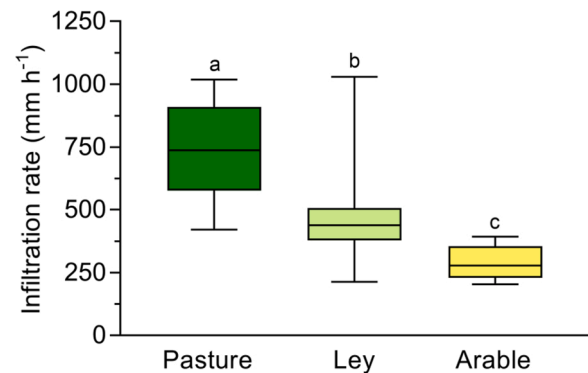
**Fig. 8.** Soil hydrological functioning as (a) water holding capacity (WHC) of entire monoliths, and (b) saturated hydraulic conductivity ( $K_s$ ) measured on 2–7 cm depth permeameter samples. Box plots show median, interquartile range, maximum and minimum values. Replication per treatment: pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ). Where bars share the same letter code they are not significantly different in Tukey post-hoc tests ( $P > 0.05$ ).

conventionally managed and intensively cultivated and cropped arable land in Yorkshire, UK, initiated multifunctional improvements in topsoil quality that in the main supported our hypotheses, with increased wheat yields, better hydrological functioning, and recovery towards the properties of pasture soils. Our findings reveal a set of important soil quality indicators that can quickly recover on arable to ley conversion, including earthworm numbers and biomass, surface soil infiltration rates, macropore flow rates, surface soil bulk density and wheat crop yields (including crop resilience to flooding).

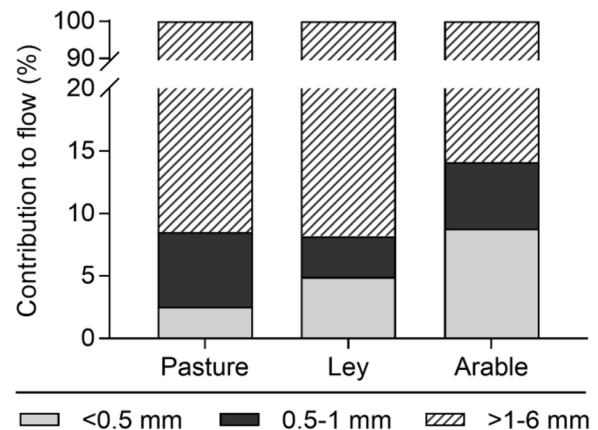
Strong linear relationships between wheat yields and soil organic carbon, total nitrogen, water-holding capacity, and BD (Fig. 12) confirmed that the yields in the arable soils are constrained by poor soil quality. With the exception of earthworm numbers, which tended to be higher in the leys than in the pasture soils, the mean values of soil properties in the leys (whether statistically significantly different to arable or not) were intermediate between those of the arable and pasture monoliths. This supports the hypothesis that the leys are starting to regenerate key soil properties and functions required for good crop yields and improved resilience to extreme weather, but this is far from complete after only 19 months. Further improvements would be expected if the leys were maintained for 3 years, as was traditional practice in the UK until the middle of the 20<sup>th</sup> Century (Johnston et al., 2017).

#### 4.2. Effects of leys on resilience to weather-stress

The unusually dry April in 2017 immediately preceding the weather-stress treatments, meant that ambient treatment experienced a moderate drought, and the improvement of wheat growth in the flooded treatment was likely due to no water-limitation. We saw no evidence of flood induced stress which can arise from anoxic conditions in the rhizosphere preventing root respiration and generating toxins (Hossain and Uddin, 2011; Herzog et al., 2016). The high resistance to flooding we found may



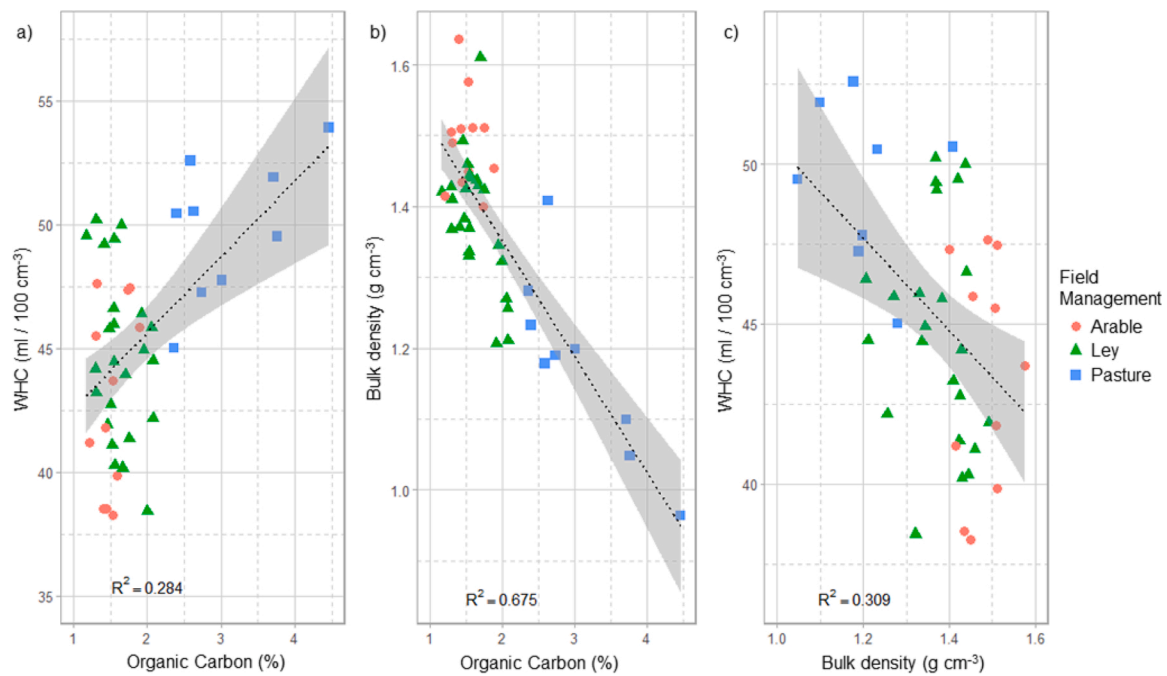
**Fig. 9.** Infiltration rate measured at the soil surface through < 6 mm sized pores. Infiltration rates through <1 mm or <0.5 mm pore sizes are not presented as no significant differences between treatments were shown. Box plots show median, interquartile range, maximum and minimum values. Replication: pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ). Different letters denote land use treatments which are significantly different ( $P < 0.05$ ).



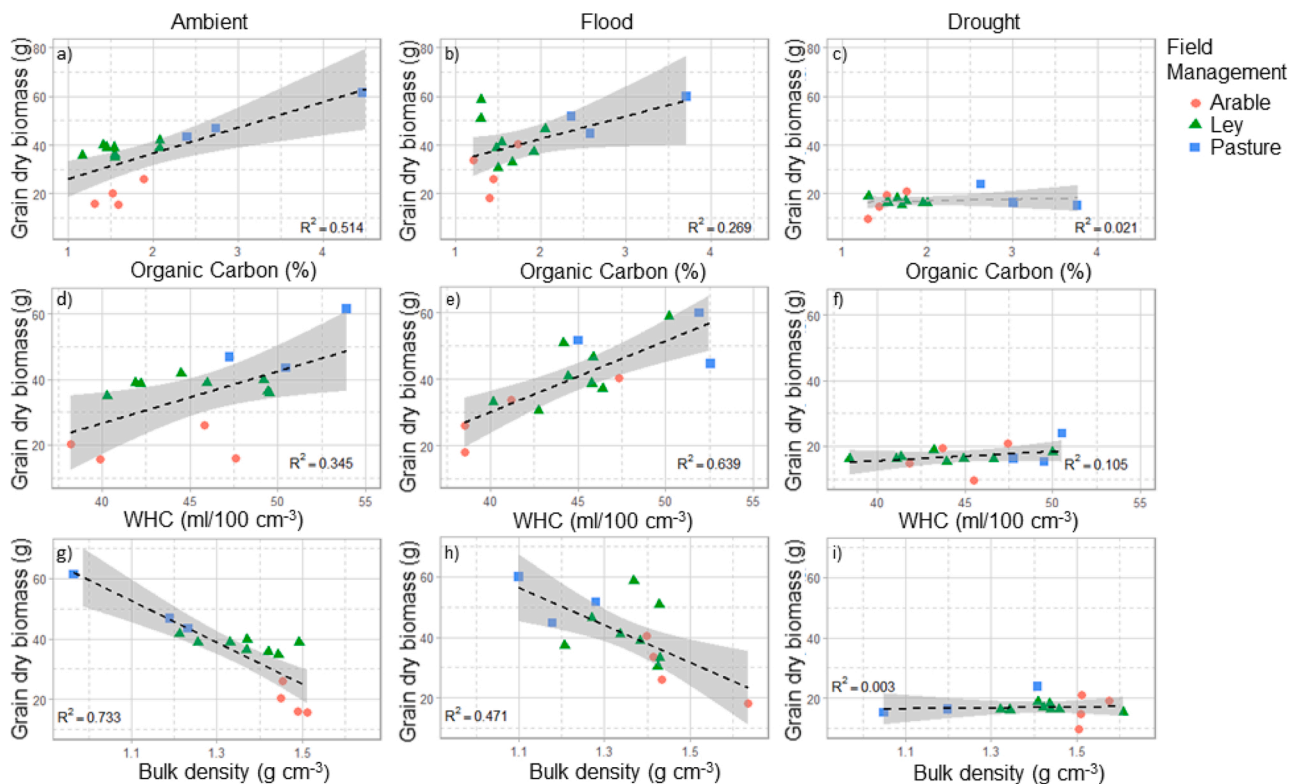
**Fig. 10.** Mean proportion of water flow through each pore size class by land use; pasture, ley and arable, measured at the soil surface. The proportion of flow through the <0.5 mm pore sizes was significantly larger in arable soils compared to pasture and ley soils ( $P < 0.001$ ). Replication: pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ).

have been because the wheat leaves were above water at stem elongation and able to supply oxygen to roots (Pedersen et al., 2021).

In the ambient and drought treatment, as the monoliths were only 22 cm deep the volume of accessible soil water will be less than in the field where wheat can sometimes root to 1 m depth (AHDB, 2018a). In the fields we sampled, bedrock occurs between 50–90 cm depth, and due to subsoil compaction, which is widespread in arable soils, wheat rooting densities rarely exceed 1 cm cm<sup>-3</sup> below the plough-layer (20–30 cm) giving low efficiencies of subsoil water and nutrient uptake (Hodgkinson et al., 2017; Clarke et al., 2017). However, under field conditions wheat may achieve deeper rooting into ley and pasture soils, down vertical burrows of anecic earthworms like *Lumbricus terrestris* which are more abundant in grasslands and untilled soils than intensively tilled arable fields (Edwards and Loft, 1982; Holden et al., 2019). The imposed drought stunted the wheat and caused leaf curling and wilting (Fig. 2; Supporting Information Figs. S4, S5). The overall increase in wheat biomass and grain production in the leys compared to the arable treatments (Fig. 4a) was clearly attributable to improved resilience to both flood and ambient (moderate drought) conditions, since there was no benefit of the ley topsoil quality improvements under extreme drought.



**Fig. 11.** Linear relationships with 95 % confidence intervals (shaded) between (a) soil water-holding capacity (WHC) and organic carbon ( $y = -2.32 + 0.09 x$ ,  $R^2 = 0.284$ ,  $P < 0.001$ ), (b) bulk density and organic carbon ( $y = 7.535 - 4.14 x$ ,  $R^2 = 0.675$ ,  $P < 0.001$ ), and (c) WHC and bulk density ( $y = 2.24 - 0.01 x$ ,  $R^2 = 0.309$ ,  $P < 0.001$ ), in arable, ley and pasture monoliths. Shaded grey areas shows 95 % confidence interval around fitted lines. Replication: pasture ( $n = 9$ ), ley ( $n = 24$ ), arable ( $n = 12$ ).



**Fig. 12.** Linear relationships with 95 % confidence intervals (shaded) between soil quality variables and wheat grain yield in response to arable, pasture land use in combination with weather-stress treatments. Wheat yields showed significant linear regressions with soil organic carbon in (a) ambient ( $y = 15.35 + 10.56 x$ ,  $R^2 = 0.514$ ,  $P = 0.002$ ) and (b) flood ( $y = 23.95 + 9.24 x$ ,  $R^2 = 0.270$ ,  $P = 0.047$ ) treatments, but not in (c) drought treatment ( $R^2 = 0.021$ ,  $P > 0.05$ ). Wheat yields showed significant linear regressions with soil water holding capacity (WHC) in (d) ambient ( $y = -36.85 + 1.58 x$ ,  $R^2 = 0.345$ ,  $P = 0.021$ ) and (e) flood ( $y = -55.48 + 2.14 x$ ,  $R^2 = 0.639$ ,  $P < 0.001$ ) treatments, but not in (f) drought treatment ( $R^2 = 0.105$ ,  $P > 0.05$ ). Wheat yields showed significant linear regressions with bulk density in (g) ambient ( $y = 128.26 - 68.86 x$ ,  $R^2 = 0.733$ ,  $P < 0.001$ ) and (h) flood ( $y = 123.79 - 61.4 x$ ,  $R^2 = 0.471$ ,  $P = 0.005$ ) treatments, but not in (i) drought treatment ( $R^2 = 0.003$ ,  $P > 0.05$ ).

#### 4.3. Grass-clover leys as biological drivers of arable soil structural regeneration

The improvements in topsoil properties, and resilience to flooding and moderate drought seen in the leys demonstrates the importance of biology in regeneration of degraded arable soils. Of particular importance are the changes to topsoil structure driven by the plants and soil organisms. Both clover species included in our leys have been shown to improve topsoil structure and soil macropores (Mytton et al., 1993; Jarvis et al., 2017), complementing the activities of earthworm burrowing (Spurgeon et al., 2013). Increases in both root biomass and earthworm populations are associated with increased infiltration rates and macropores flows within pasture soils (Weiler and Naef, 2003; Fischer et al., 2014; Hallam et al., 2020) and are likely to be important in the effects of the leys on soil quality and hydrological functioning. Both earthworms (Crittenden et al., 2014) and mycorrhizal fungi benefit from ceasing ploughing, and help improve soil aggregation, reduce soil BD and contribute to sequestering SOC (Wilson et al., 2009; Zhang et al., 2013; Sokol et al., 2018). The lower soil BD and better water holding capacity seen in pasture and ley soils compared to arable soil in our study have previously been found to be positively associated with increased earthworm abundance (Crittenden et al., 2014), reflecting positive feedbacks since earthworms can drive these changes, as shown experimentally at our field site (Hallam et al., 2020). The initial earthworm population sizes and soil conditions, which will depend on previous land management history and soil type, are therefore likely to affect the rate of recovery of earthworms and soil structure and functional improvements under the leys.

The regenerative effects of leys on degraded intensively cultivated arable soils is shown to provide important societal benefits such as increased rates of water infiltration through macropores which will be beneficial for reducing overland flow during rainfall events as well as increasing crop water availability (Obour et al., 2018). The increase in water-storage capacity of the leys compared to the arable soil was reflected in requirement for an extra 1.85 mL 100 cm<sup>-3</sup> in order to achieve the same depth of flooding, which increased to 4.24 mL 100 cm<sup>-3</sup> in the pasture soils indicative of an increase in soil porosity (Fig. 1). These effects are consistent with decreasing soil bulk density in the ley. If leys were widely re-introduced as part of arable rotations they may help reduce flood and soil erosion risks exacerbated by arable soil structural degradation and climate change (Nearing et al., 2004). For example, over a third of the area of the Ouse catchment in Yorkshire, from which our soils were taken suffers structural degradation (Holman et al., 2003), and over 31,000 properties are at risk of flooding in this catchment from extreme rainfall events that are increasing in frequency (Environment Agency, 2010). Consequently, despite the central importance of SOC for soil functioning (Graves et al., 2015; Lal, 2018) to understand the benefits of reintroducing leys into arable rotations, especially in early years following conversion requires a broader suite of functional measurements to be made, as in this study.

#### 4.4. Earthworm population recovery under leys

The increase in quantity and persistence of organic carbon and nitrogen inputs into soil by evergreen grass-clover leys (McNally et al., 2015), compared to arable crops like wheat (Sun et al., 2018) together with ceasing mechanical disturbance by tillage, provides a much more favourable environment for earthworms and for regenerating soil structure and functions. This is supported by a meta-analysis of 16 studies of effects of arable to pasture conversion upon earthworm communities which found (means and standard errors respectively), 56.3 ± 70.8 (sem) individuals m<sup>-2</sup> in arable land and 229 ± 193 individuals m<sup>-2</sup> in pasture (Spurgeon et al., 2013) and larger earthworm populations under less intensive tillage management (Hendrix et al., 1992). By comparison, we found higher numbers with lower variance (146.1 ± 26.0 individuals per m<sup>-2</sup> in arable soil monoliths and 263.2 ±

47.1 individuals per m<sup>-2</sup> in monoliths from pasture). However, these values are lower than in surveys conducted in April 2015–17 in the same fields from which the monoliths were taken, where there were 325.5 ± 254.7 individuals per m<sup>-2</sup> in arable and 757.5 ± 426.2 individuals per m<sup>-2</sup> in pasture land (Holden et al., 2019). Nonetheless, all these studies show reduced earthworm populations in arable compared to pasture soils.

Differences in earthworm numbers between the studies may be caused by time of year, sampling methods, and soil depth. In the field, allyl isothiocyanate solution was used to extract earthworms from below 18 cm depth (Holden et al., 2019), while the monoliths only extended to 22 cm. Monoliths did not contain any *Lumbricus terrestris* which produce vertical burrows to about 1 m depth, and contributed >10 % of total earthworm biomass in both the arable and pasture fields (Holden et al., 2019). The absence of anecic species like *L. terrestris* in our study may have been because trenching around the monoliths in the field caused these earthworms to retreat into deep burrows below the sampled depth. Seasonality is important, as six year study of pasture woodland by Eggleton et al. (2009) found highest earthworm abundance in March and lowest in September, when we sampled, whereas Holden et al. (2019) sampled in April, close to the annual peak. Although the diquat herbicide used treat the pasture and ley monoliths is moderately toxic to earthworms (Syngenta, 2007), there was no evidence that this decreased the earthworm numbers relative to untreated arable soil monoliths.

The absence of significant weather-stress treatment effects on the earthworm populations in the monoliths was surprising, especially for the drought treatment. However, some earthworms can survive droughts by becoming quiescent or generating aestivation chambers and entering diapause dormancy which slows desiccation (Evans and Guild, 1948; Holmstrup, 2001). Earthworms reproduce by cocoons that can undergo dormancy until conditions are appropriate. It is likely that with the dry conditions in April before the weather-stress treatment many of the earthworms had produced cocoons that are likely to have hatched after the rewetting of the soil in late May. Longer periods of water limitation extending over several months reduce earthworm abundance (Hendrix et al., 1992; Eggleton et al., 2009) and acute drought of 14 days with soil water contents below 10–12 % seriously impacted subsequent reproductive activity of *Allobophora caliginosa* (Holmstrup, 2001). In relation to flooding, Ausden et al. (2001) found common UK earthworm species, including *Allobophora chlorotica* which accounted for 65 % of the earthworms recorded in the monoliths, could survive over 120 days of constant submergence in aerated water. The regular addition of water to the flooded monoliths to replace evapotranspiration losses may have helped keep the water sufficiently oxygenated for the earthworms to survive.

#### 4.5. Effects of leys on soil organic carbon sequestration

Short-term studies of leys frequently report increases in SOC that are not statistically significant, because of the inherent variability of soils and the initial increases being small relative to the existing soil carbon stocks. For example, Puerta et al. (2018) found soil organic carbon 0–6 cm deep increased on average 8% with two year grass-clover leys following conventional arable cropping with intensive tillage or no-tillage for 4 years, but this was not significant. Gosling et al. (2017) also reported a non-significant 5% increase in SOC to 10 cm depth after one or two years of grass compared to permanent intensive arable cultivation. The non-significant 7.6 % increase in SOC we found in the 19 month ley following decades of intensive arable cultivation (Fig. 6a) is consistent with these previous short-term studies. Furthermore, Puerta et al. (2018) showed that leys regenerate macroaggregates which protect organic C and N and enable their sequestration, often at fairly constant C:N ratios, consistent with the constant soil C:N ratios we report across a range of SOC concentrations from arable, ley and pasture soils (Fig. 6c). The importance of biology in driving soil aggregation and C sequestration is corroborated by three times higher rates of SOC



accumulation found in surface soils of leys in organic compared to conventional arable land (Puerta et al., 2018).

Longitudinal studies show the rates of C accumulation seen in short-term studies of leys give statistically significant increases in SOC stocks over longer periods (Kirk and Bellamy, 2010; Poeplau and Don, 2015; Prade et al., 2017). In their 68 year study of effects of 3 year grass-clover leys in rotations with 2 years of arable cropping, Johnston et al. (2017) showed SOC on a sandy loam soil increased from 0.98 % under long-term arable cropping to an equilibrium value of about 1.3 % with the leys, over a period of about 30 years. Given the strong positive correlation between SOM content and water-holding capacity (Hudson, 1994), these increases have important implications for hydrological functioning as well as carbon sequestration at larger spatial and temporal scales than studied in our experiment.

#### 4.6. Strengths and limitations of the monolith approach

The monolith approach allowed drought and flood treatments and detailed evaluation of the hydrological functioning of the topsoil (to 22 cm) in response to the leys, as well as quantifying a wide range of important soil properties and functions including an assessment of crop performance in response to arable to ley conversion. However, the shallow depth of the monoliths, lack of root access to subsoil, and lack of capillary connections between topsoil and subsoil, mean the findings are unlikely to fully reflect *in situ* crop responses in the field. Furthermore, the transplanting of wheat seedlings rather than being grown from seeds is likely to have resulted in some seminal root damage, and the small sizes of the monolith boxes, although placed together in a block to create a continuous crop canopy, may have suffered edge effects on the yields, despite being in a relative sheltered outdoor compound. We are conducting follow-up field trials to assess *in situ* the effects of reintroducing legume-rich leys into arable rotations on wheat performance under conventional tillage and direct drilling, to address these issues.

## 5. Conclusions

Reintroduction of grass-clover leys for only 19 months into intensively cultivated, organic matter depleted, arable fields started to restore topsoil hydrological functioning, wheat yields and crop resilience to flooding and moderate drought towards those of permanent grasslands.

Our study identifies a set of indicators of soil qualities that deliver important multifunctional benefits and can quickly recover on arable to ley conversion, including earthworm numbers and biomass, surface soil infiltration rates, macropore flow rates, surface soil bulk density and wheat crop yields (including crop resilience to flooding and moderate drought). Whilst previous studies have tended to focus on potential increases in SOC due to leys, the rapid improvements we have found in topsoil structure and hydrological functioning demonstrate a wider set of societal benefits that could justify payments under public money for public goods arrangements as is being proposed in the UK Environmental Land Management Scheme (Defra, 2018). Further studies are needed to establish the extent to which leys maintained for 2–4 years continue to regenerate arable soil quality, and whether the effects on the Cambisol we studied extend to other soil types.

#### Author contributions

JRL designed and led the study. LGF and MGL established the ley strips, and DB, MGL, SPFH, MEH, PJC, and AT extracted and transported the monoliths. AT devised the open sided-polytunnel and temporary sealing of boxes to flood monoliths, and assisted DB in data collection. RPG and JH guided the infiltrometry. JL, supervised by PJW, undertook the earthworm population studies. DB collected and analysed the data and wrote the paper with JRL, with comments from the other authors.

## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.still.2021.105037>.

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