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Guest, Emily J, Palfreeman, Lucy J, Holden, Joseph et al. (5 more authors) (2022) Soil macroaggregation drives sequestration of organic carbon and nitrogen with three-year grass-clover leys in arable rotation. *Science of the Total Environment*. ISSN: 0048-9697

<https://doi.org/10.1016/j.scitotenv.2022.158358>

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# Soil macroaggregation drives sequestration of organic carbon and nitrogen with three-year grass-clover leys in arable rotations

Emily J. Guest<sup>a,e,\*</sup>, Lucy J. Palfreeman<sup>a</sup>, Joseph Holden<sup>b</sup>, Pippa J. Chapman<sup>b</sup>, Les G. Firbank<sup>c</sup>, Martin G. Lappage<sup>c</sup>, Thorunn Helgason<sup>d,f</sup>, Jonathan R. Leake<sup>a</sup>

<sup>a</sup> Plants, Photosynthesis and Soil, School of Biosciences, The University of Sheffield, Sheffield S10 2TN, UK

<sup>b</sup> School of Geography, The University of Leeds, Leeds LS2 9JT, UK

<sup>c</sup> School of Biology, The University of Leeds, Leeds LS2 9JT, UK

<sup>d</sup> Department of Biology, The University of York, York, YO10 5DD, UK

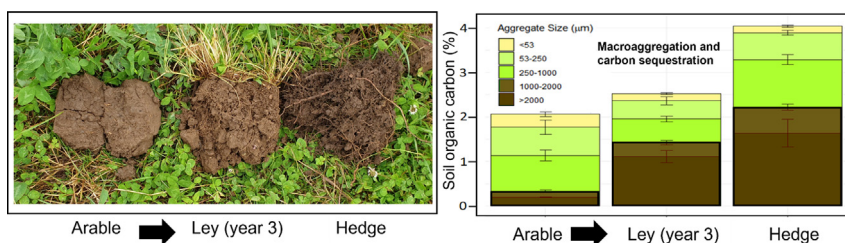
<sup>e</sup> ADAS Boxworth, Battle Gate Road, Cambridge CB23 4NN, UK

<sup>f</sup> School of Biological Sciences, The University of Edinburgh, Edinburgh, EH9 3JR, UK

## HIGHLIGHTS

- Three-year leys increased macroaggregates >5-fold in a long-term arable rotation.
- Soil organic C and N increased through accumulation in macroaggregates.
- Macroaggregate-stored organic C is a key indicator of improving soil health.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Wei Shi

### Keywords:

Soil health

Water-stable aggregates

Regenerative agriculture

Soil organic matter

## ABSTRACT

Conventional arable cropping with annual crops established by ploughing and harrowing degrades larger soil aggregates that contribute to storing soil organic carbon (SOC). The urgent need to increase SOC content of arable soils to improve their functioning and sequester atmospheric CO<sub>2</sub> has motivated studies into the effects of reintroducing leys into long-term conventional arable fields. However, effects of short-term leys on total SOC accumulation have been equivocal. As soil aggregation may be important for carbon storage, we investigated the effects of arable-to-ley conversion on cambisol soil after three years of ley, on concentrations and stocks of SOC, nitrogen and their distributions in different sized water-stable aggregates. These values were benchmarked against soil from beneath hedgerow margins. SOC stocks (0–7 cm depth) rose from 20.3 to 22.6 Mg ha<sup>−1</sup> in the arable-to-ley conversion, compared to 30 Mg ha<sup>−1</sup> in hedgerows, but this 2.3 Mg ha<sup>−1</sup> difference (or 0.77 Mg C ha<sup>−1</sup> yr<sup>−1</sup>) was not significant). However, the proportion of large macroaggregates (> 2000 µm) increased 5.4-fold in the arable-to-ley conversion, recovering to similar abundance as hedgerow soils, driving near parallel increases in SOC and nitrogen within large macroaggregates (5.1 and 5.7-fold respectively). The total SOC (0–7 cm depth) stored in large macroaggregates increased from 2.0 to 9.6 Mg ha<sup>−1</sup> in the arable-to-ley conversion, which no longer differed significantly from the 12.1 Mg ha<sup>−1</sup> under hedgerows. The carbon therefore accumulated three times faster, at 2.53 Mg C ha<sup>−1</sup> yr<sup>−1</sup>, in the large macroaggregates compared to the bulk soil. These findings highlight the value of monitoring large macroaggregate-bound SOC as a key early indicator of shifts in soil quality in response to change in field management, and the benefits of leys in soil aggregation, carbon accumulation, and soil functioning, providing justification for fiscal incentives that encourage wider use of leys in arable rotations.

\* Corresponding author presently at: ADAS Boxworth, Battle Gate Rd, Cambridge, CB23 4NN, UK.

E-mail addresses: [emily.guest@adas.co.uk](mailto:emily.guest@adas.co.uk) (E.J. Guest), [lucy.palfreeman@gmail.com](mailto:lucy.palfreeman@gmail.com) (L.J. Palfreeman), [J.Holden@leeds.ac.uk](mailto:J.Holden@leeds.ac.uk) (J. Holden), [P.J.Chapman@leeds.ac.uk](mailto:P.J.Chapman@leeds.ac.uk) (P.J. Chapman), [L.Firbank@leeds.ac.uk](mailto:L.Firbank@leeds.ac.uk) (L.G. Firbank), [M.G.Lappage@leeds.ac.uk](mailto:M.G.Lappage@leeds.ac.uk) (M.G. Lappage), [thorunn.helgason@ed.ac.uk](mailto:thorunn.helgason@ed.ac.uk) (T. Helgason), [j.r.leake@sheffield.ac.uk](mailto:j.r.leake@sheffield.ac.uk) (J.R. Leake).

## 1. Introduction

Sustainable management of agricultural soils is essential to maintain the natural capital and ecosystem services that healthy soils can provide (Johnston and Poulton, 2018; Smith et al., 2015). These include producing high quality crops, storage of soil organic carbon (SOC), nutrients and water, infiltration and filtration of rainwater benefitting surface and groundwater quality, all of which are vital to meet societal needs (Blum, 2005; Brevik et al., 2018). The requirement to manage soil well to maintain organic matter content, structure, and associated hydrological functioning is even more critical as a result of climate change (IPCC, 2019) increasing frequency and intensity of both drought and extreme rainfall events (Groisman et al., 2005; Samaniego et al., 2018). This increases risks of crop failures, flooding, and soil erosion (Nearing et al., 2004), with serious environmental and human consequences that often exacerbate social inequalities (Sayers et al., 2017). Soil hydrological functioning, and especially water infiltration, percolation and throughflow, are critically dependent on macropores and structural stability on wetting to maintain functional macroporosity (Xiao et al., 2017). If soil disaggregates on rainfall impact or wetting this results in plugging of pore spaces and the generation of surface capping which impedes infiltration and leads to infiltration-excess overland flow (Holden, 2020). This preferentially carries away the finer and lighter soil fractions such as clays and organic matter, further degrading soil quality and functions (Xiao et al., 2017; Yang et al., 2020). Water-stable aggregates (WSA) are therefore a key determinant of soil quality and structure (Churchman, 2010), and also play a central role in carbon (C) accumulation (Tisdall and Oades, 1982; Jastrow, 1996; Six et al., 1998, 2004; Stewart et al., 2008, 2009). These crumb-like particles are conventionally grouped into size categories, with the smallest-sized fraction (<53  $\mu\text{m}$ ) containing silt and clays that sometimes form very small microaggregates. The middle-sized fraction ranges from 53 to <250  $\mu\text{m}$  and comprises microaggregates, while the macroaggregate fraction that joins together silt and clay particles, microaggregates, and organic matter ranges from 250- >2000  $\mu\text{m}$ , with those >2000  $\mu\text{m}$  being defined as large macroaggregates (Six et al., 2000; Totsche et al., 2018).

According to the hierarchical model of aggregate formation proposed by Tisdall and Oades (1982), microaggregates are bound together by persistent binding agents, and then enmeshed together in groups by temporary and transient binding agents, such as roots and fungal hyphae, to form macroaggregates. An important recent advance in understanding these processes has been the evidence that SOC accumulation is driven by microbial interactions with mineral surfaces (Kallenbach et al., 2016; Liang et al., 2017). This appears to result from microbially-derived organic carbon (OC) being bound to mineral surfaces within microaggregates that then protect it from decomposition within the larger macroaggregates (Yu et al., 2015) that are assembled mainly by the actions of roots and soil organisms (Six et al., 2000; Denef et al., 2004; Briedis et al., 2012). This directly links the processes of C accumulation to the assembly of macroaggregates by soil organisms, and to the field management practices that impact the ecosystem engineer organisms such as mycorrhizal fungi and earthworms delivering these functions.

As the binding agents that impart structural stability to macroaggregates are labile and their turnover is accelerated by tillage (Tisdall and Oades, 1982; Portella et al., 2012; Rillig et al., 2015), arable cropping normally increases the accessibility and degradation of SOC by soil microorganisms (Ye et al., 2020). Intensification of arable production in the past few decades, for example in Europe, has focussed on short rotations with the most profitable winter cereal and oilseed crops that were established by annual ploughing and harrowing (Townsend et al., 2016; Wezel et al., 2014). These intensive short rotations have depleted SOC and weakened soil structures, increasing disaggregation, slumping and compaction (Haghighi et al., 2020; Pires et al., 2017). Crop selection for enhanced partitioning of organic matter (OM) into above-ground biomass (AGB) compared to below-ground, and removal of this AGB during harvesting, has contributed to soil degradation through reduced OM inputs. For example, the most widely grown crop in the UK, wheat, allocates about

50 % of its AGB into grain (AHDB, 2018), the remainder being straw and chaff. Only about 50 % of cereal straw is added to the soil in the UK (Townsend et al., 2018). The increasing market for straw for livestock bedding and electricity generation in purpose-built power stations has contributed to more than a third of straw being sold from UK arable farms, rather than being left as residues within the field (Townsend et al., 2018).

OM inputs to arable soils have also fallen with intensification due to lower inputs of C from root turnover and root exudates. For example, modern wheat varieties contribute as little as 0.4  $\text{Mg C ha}^{-1} \text{yr}^{-1}$  to soil via roots (Sun et al., 2018), compared to around 1  $\text{Mg ha}^{-1} \text{yr}^{-1}$  under a ryegrass-clover sward (McNally et al., 2015). Living roots disproportionately contribute to SOC accumulation relative to litter inputs from AGB, due to higher chemical recalcitrance of root tissues and microbial protection, especially in deeper soil horizons (Rasse et al., 2005; Sokol et al., 2019). Consequently, the 20th century shift away from mixed farming in which grass-clover leys typically contribute 2.5 times more C inputs to soil via roots than wheat (McNally et al., 2015) and receive C return of AGB via manure inputs from livestock, is strongly implicated in the decline in arable SOC (King et al., 2005; Kirk and Bellamy, 2010).

Roots and manures have been shown to be important in promoting soil aggregation, C accumulation, and supporting earthworm populations (Tiwari, 1993), which burrow and ingest soil, and release worm-casts. Together, roots of perennial grasses and clovers, and earthworms, interact to generate macroaggregates and macropores, improving soil hydrological functioning and crop growth (Hallam et al., 2020; Hallam and Hodson, 2020). Conversion of intensively cultivated arable land to short term legume-rich leys has been found to lead to a rapid recovery in earthworm populations (Prendergast-Miller et al., 2021) and to major improvements in soil hydrological functioning, including increased pore space and reduced compaction (lower bulk density), faster infiltration rates via macropores and higher saturated hydraulic conductivity (Berdeni et al., 2021). These biological and structural improvements in soil quality have been correlated with large increases in wheat crop yield resilience to flooding and moderate drought (Berdeni et al., 2021). However, despite the importance of SOM for soil structure and aggregate stability (Li et al., 2021), increases in total SOC by 19 month old leys followed by a direct drilled wheat crop, studied by Berdeni et al. (2021) were not statistically significant, but nonetheless are strongly implicated in the large biological, soil structural, and crop performance improvements seen.

Several recent studies corroborate that although total SOC tends to increase under two-three-year leys introduced into arable rotations, over these short time periods the increases are typically not statistically significant (Gosling et al., 2017; Puerta et al., 2018). In the former case it was concluded that arable to ley conversion is a poor candidate for meeting C sequestration targets in Europe (Gosling et al., 2017). However, detecting statistically significant short-term changes in bulk SOC is constrained by the inherent spatial variability of soils, and the amount accumulated over the ley period will be small compared to the existing stock, but may nonetheless still make important contributions to improving soil functions and health, including C sequestration. This is clearly shown in multi-decadal studies of leys in arable rotations demonstrating significantly higher SOC concentrations that are important for C storage at landscape scales over 50–70 years (Jarvis et al., 2017; Johnston et al., 2017; Poeplau and Don, 2015; Prade et al., 2017). Similarly, an overview of multi-decadal studies shows substantial increases in the rate of SOC sequestration in the early years after management change, including the incorporation of arable/ley rotations, organic amendments or conversion to grassland (Poulton et al., 2018). Thereafter, C accumulation rates decline as they progress towards a new saturation-equilibrium (Poulton et al., 2018). For example, Baveye et al. (2018) present data from the USA where arable to grassland conversion at several sites gave initial rates of SOC accumulation of 0.8  $\text{Mg ha}^{-1} \text{yr}^{-1}$ , but this rate declined exponentially halving every decade, and approached saturation after 50 years. The frequent lack of statistically significant annual increases in bulk SOC at the point in time when these are occurring at their fastest rates, such as in arable to grassland conversion, indicates the need to develop more effective measures to guide our



understanding of both the processes and rates of C accumulation in the first two-three years of arable to ley and arable to grassland conversions, and for early evaluation of effectiveness management practices to facilitate C accumulation.

One promising approach is to link studies of SOC accumulation to the processes of soil aggregation, which protects this C, by fractionating soil into WSA of different sizes and quantifying the OC pools in these fractions. A recent study of a sandy-silt cambisol on an arable-to-ley conversion in a field under conventional and organic management in Switzerland, showed strong preferential accumulation of C in large ( $>2000\ \mu\text{m}$ ) macroaggregates in soil 0–6 cm depth (Puerta et al., 2018). In that study, the two-year leys in the conventional and organic rotations that had previously experienced intensive tillage and were frequently mown and highly fertilized with cattle slurry ( $205\ \text{kg N ha}^{-1}\ \text{y}^{-1}$ ) showed a significant increase (by 65 and 47 %, respectively) in WSA  $>2000\ \mu\text{m}$ . This increased the contribution of these large macroaggregates to total soil C. Previous studies have suggested that N-fixing legumes such as clovers co-sown with grasses increase SOC relative to grass-only swards (Poulton et al., 2018), and there are some indications that co-storage of SOC and N may be linked via constrained C:N ratios (Berdeni et al., 2021).

Here we set out to test the hypothesis that the introduction of a three-year grass-clover ley into replicated long-term ( $>20$  years), intensively cultivated arable fields on a silty cambisol in the UK increases the proportion of large soil macroaggregates and the storage of OC and N within them.

Our study focussed on four conventionally managed arable fields in which leys were sown in strips in the NERC-funded Soil Security Programme project SoilBioHedge. Arable management continued alongside the leys so that soil could be sampled and compared at the same time for both arable and ley treatments in the same fields. The aims of the study were to assess how the mown, but not fertilized, three-year grass-clover leys influence soil aggregation through the measurement of aggregate size distribution and resulting changes to the distribution of OC and N in these fractions, with the bulk soil total OC and N values being determined by summing the fractions. A previous study on the same soils found modest but not significant increases in bulk SOC and N after 19 months of arable-to ley conversion, followed by a wheat crop (Berdeni et al., 2021). Here, we aim to determine how the distribution of OC and N in soil aggregates changes after three years of arable-to-ley conversion, testing the prediction of improved soil macroaggregation resulting in the protection and accumulation of OC and N within macroaggregates. We compared the leys to the arable control and hedgerow margin soils as benchmark start and endpoint comparisons for C saturation potentials, and to evaluate the extent of recovery of structure and chemistry of the soil under ley relative to permanently uncultivated hedgerow soils. Our approach uses a ‘space for time’ substitution approach whereby differences in soil properties in the leys compared to the adjacent arable controls sampled at the same time are interpreted as change that has occurred over the three years of the leys. This is supported by results from the wider research team working on the SoilBioHedge experiment, of which the present study is part of, as well as other published research (Johnston et al., 2017; Baveye et al., 2018), which evidence little variation in long-term arable fields after this duration of establishment.

## 2. Methods

### 2.1. Field site and experimental design

Paired grass-clover ley strips (3 m wide, 70 m long and 48 m apart) were sown into four arable fields at The University of Leeds Farm, Tadcaster ( $53^{\circ}52'25.2''\text{N}\ 1^{\circ}19'47.0''\text{W}$ ) in May 2015, to study spatial and temporal changes in soil quality (Fig. 1). The soils in these fields are Calcaric Endoleptic Cambisols (IUSS Working Group WRB, 2015) but differ slightly in textural class, with BSSW and BSSE being silt loams and Copse and Hillside being loam and sandy loam, respectively, with the sand content ranging from 41.3 % ( $\pm 0.8$ ) in BSSW, to 47.9 % ( $\pm 2.5$ ) in Copse (Hallam et al., 2020). The ley seed mix comprised diploid and tetraploid

*Lolium perenne* (20 %, and 16 %, respectively), *Festulolium* spp., 16 %, two varieties of tetraploid *Lolium x boucheanum* (12 % and 16 %), *Trifolium repens* 5 %, and *Trifolium pratense* 15 %, at an overall seeding rate of  $4.2\ \text{g m}^{-2}$ . The ley strips were mown four times per year, with grass clippings removed from the leys from June 2016 onwards. In April and May 2016, clippings were returned to the ley.

The four fields had been under continuous arable rotations and ploughed annually since they were last under ley in 1988 (Copse) and 1994 (Big Substation West (BSSW) and Big Substation East (BSSE)). One field had been in grassland for 11 years until 2009 (Hillside). Since 2009, the three fields were used to grow potatoes, winter wheat, oilseed rape, vining peas, winter barley and spring barley under conventional intensive management. During the three years (2015–2018) in which the leys were grown in these fields the rest of the field area was used to grow spring barley, winter barley and oilseed rape respectively. The arable parts of the fields, but not the leys, received typical fertiliser inputs of  $140\text{--}150\ \text{kg N ha}^{-1}$ ,  $70\text{--}86\ \text{kg K ha}^{-1}$ ,  $23\ \text{kg P ha}^{-1}$ ,  $22\ \text{kg S ha}^{-1}$ , and in autumn 2017 received  $8\ \text{Mg ha}^{-1}$  of pig manure. They also received standard applications of fungicides (Prothioconazole seed dressing, Chlorothalonil, Cyprodinil, Epoxiconazole, Fenpropimorph, Fluoxastrobin, Fluxapyroxad, Metconazole, Proquinazid, and Tebuconazole), herbicides (Glyphosate, Diflufenican, Flufenacet, Fluroxypyr, Metsulfuron-methyl, and Tribenuron-methyl), insecticides (Clothianidin seed dressing on barley and wheat, Cypermethrin, Lambda-cyhalothrin and Pirimicarb), molluscicide (Metaldehyde) and cereal growth regulators (Chlormequat, and Trinexapac-ethyl).

The paired ley strip design enabled direct comparison of the changes to soil properties under ley compared to conventionally tilled and intensively cropped arable rotations at the same time, and at the same distance from the field margins. One of each ley pair (Fig. 2) was continuous to the field margin (CAL). The other (UAL) was separated by a 2 m wide fallow strip parallel to the hedges, 13 m long and extending 5 m to the left and right of the ley and along which a vertical stainless steel mesh curtain barrier was inserted to the bedrock at about 90 cm depth (Berdeni et al., 2021). This arrangement was designed to prevent the recruitment of earthworms and mycorrhiza from the field margin in the original SoilBioHedge project design.



Fig. 1. Map of The University of Leeds Farm, Tadcaster, showing the four sampling fields: Copse, Hillside, BSSE and BSSW. The 70 m long, green paired ley strips are visible in each field from a Google Earth satellite picture, taken on 17/07.2017. Map data © 2017 Google.

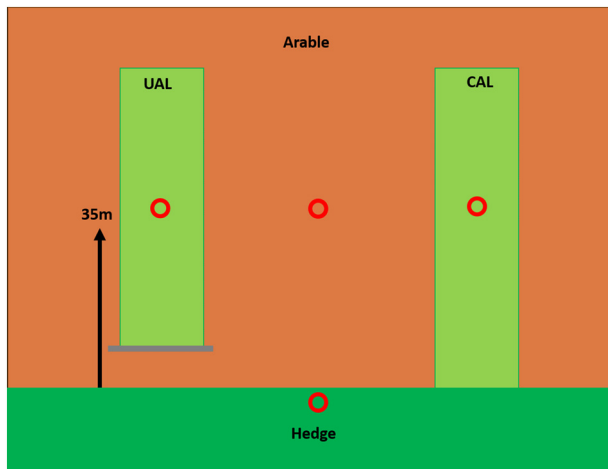


Fig. 2. Experimental design for soil collection from The University of Leeds Farm, Tadcaster. UAL = Unconnected Arable Ley, CAL = Connected Arable Ley, Open red circle = soil sample site. Not drawn to scale.

## 2.2. Characteristics of the field hedgerows

The arable fields were bounded by well-established hedgerows that are likely to be more than a century old. They were 1.8 m to 4.8 m high and 0.28 m to 1.31 m wide and managed via trimming using a tractor mounted flail mower every one to two years (Holden et al., 2019). They contained a mixture of woody species, dominated by *Crataegus monogyna* (60 %) with *Sambucus nigra* (10 %) and *Ilex aquifolium* (10 %), accompanied by <10 % cover each of *Corylus avellana*, *Cornus sanguinea* and *Rosa canina* with occasional *Prunus spinosa*, *Acer campestre*, *Fraxinus excelsior*, *Euonymus europaeus*, and *Rhamnus cathartica* (Holden et al., 2019).

## 2.3. Soil sampling

In July 2018, soil cores were taken from the top 7 cm of the soil in the middle of each ley strip, 35 m from the hedge, in the arable fields between the paired ley strips, and under the hedge between the paired ley strips (Fig. 2). All four fields were growing oilseed rape (*Brassica napus*) at the time of sampling. The cores were contained in a 5 cm deep, stainless steel ring in an Eijkkamp bulk density corer, the excess soil from a 7 cm deep core being trimmed off the top and bottom of the steel ring to give 100 cm<sup>3</sup> of soil for analysis. The soil cores were stored at 4 °C for subsequent analysis.

## 2.4. Water-stable aggregate fractionation

The fresh soil samples were gently passed through a 2 cm coarse sieve to remove any stones, earthworms, vegetation and large roots. Subsamples of 70 ± 5 g were wet-sieved by hand, using a method adapted from Elliott (1986), through a series of four sieves to obtain five WSA size fractions following Puerta et al. (2018). These were: large macroaggregates (>2000 µm), medium macroaggregates (1000–2000 µm), small macroaggregates (250–1000 µm), microaggregates (53–250 µm) and silt and clay (<53 µm). The latter fraction also contains very small microaggregates (Totsche et al., 2018). Soil was submerged with water 15 mm above the 2000 µm sieve mesh, and the sieve moved vertically back and forth 50 times over a period of 1 min and 30 s. Any floating material and stones were removed and aggregates on the sieve were washed into a pre-weighed aluminium tin. This process was repeated for the remaining soil on a 1000 µm sieve (40 strokes over 1 min 10 s), a 250 µm sieve (30 strokes over 1 min 20 s) and a 53 µm sieve (10 strokes not timed over as long as needed). All fractions were dried in a 105 °C oven for 48 h and weighed.

The following equation was used to calculate the aggregate size distribution by determining the percentage contribution of each aggregate size

fraction:  $\frac{\text{fraction weight (g)}}{\text{total sample weight (g)}} \times 100$ . The data from 'BSSW Hedge' were excluded from the statistical analyses as in these samples the contribution of the >2000 µm aggregate fraction was 10-fold lower than in the other three fields, most likely because the soil was highly disturbed by a rabbit warren with burrows that emerged under the hedge adjacent to the ley strips. This hedge was in poor condition with major gaps, so was excluded as not suitable as an end-point indicator for SOC accumulation. Instead, to ensure adequate replication of samples from typical well-established hedgerows, we included samples from the nearby field, Hillside. However, the arable and ley samples from Hillside, which had been in permanent grassland set aside and leys for 60 % of the rotation over 48 years (1970–2018) were not included in the statistical analyses as these samples showed much higher >2000 µm aggregate fraction, compared to the other three fields that had been cultivated and cropped annually for 23 years.

## 2.5. Inorganic C removal from dried aggregates

The dried aggregate fractions produced from the WSA sieving were homogenised by mortar and pestle into a fine powder and 90 ± 5 mg of each sample weighed into 1.5 ml Eppendorf tubes. To each tube was added 500 µl 6 M HCl, which was stirred using a blunt needle and left for 30 min. Additional HCl was added in increments of 100 µl and stirred until the sample stopped effervescing, indicating all reactive inorganic C had been removed. Samples were left for 24 h in a fume hood to settle, enabling the removal of the supernatant. Samples were then oven-dried at 105 °C for 24 h to remove the remaining HCl.

## 2.6. Organic carbon and nitrogen analysis of dried aggregate fractions

The dried soil samples from which inorganic-C had been removed were homogenised and 30–50 mg transferred into tin boats for analysis of OC and total N percentage and C:N ratios determined by dry combustion in a CN analyser (Vario EL Cube, Hanau, Germany) using acetanilide (3–8 mg) as a standard.

## 2.7. Organic carbon and nitrogen distribution

The aggregate size distribution data was combined with the OC% and N % raw data in the following equation:  $\frac{\% \text{weight of aggregate fraction} \times \text{nutrient content (\%)}}{100}$ . This gave a dataset of soil SOC and N concentrations, and their distribution in the different aggregate size fractions.

## 2.8. Determination of soil bulk organic carbon and nitrogen stocks

Previous studies in the same fields have revealed that surface soil bulk density can be significantly reduced after 18 months of arable to ley conversion (Berdeni et al., 2021), and is much lower in hedgerow soils than arable soils (Holden et al., 2019). Consequently, changes in SOC on a concentration basis do not accurately reflect the changes in total SOC stocks per unit soil volume or area of a field. To determine the effects of land management on the stocks of SOC from 0 to 7 cm depth, we combined the results of the present study with bulk density measurements made for the same field sites and sampled in the same year to 0–7 cm depth using the same methodology as described above for soil sampling (Shaw, 2018). This previous study had found fine earth bulk density (excluding stones >1000 µm) decreased from the arable (1.41 g cm<sup>-3</sup>; *n* = 24), to ley (1.33 g cm<sup>-3</sup>; *n* = 48) to hedge (1.06 g cm<sup>-3</sup>; *n* = 12) soils (Holden et al., 2019). Taking into account the actual mass of fine earth soil to 7 cm depth in each case, the total SOC and N in Mg ha<sup>-1</sup> in the top 7 cm of soil were calculated using the following equation (Method 2 in Poeplau et al., 2017), where  $SOC_{stock_i}$  is the SOC stock of the investigated soil layer (Mg ha<sup>-1</sup>),  $SOC_{con_{fine\ soil}}$  is the

content of SOC in the fine soil (%) and  $depth_i$  is the depth of the respective soil layer (cm).

$$BD_{\text{fine soil}} = \frac{\text{mass}_{\text{sample}} - \text{mass}_{\text{rock fragments}}}{\text{volume}_{\text{sample}} - \frac{\text{mass}_{\text{rock fragments}}}{\rho_{\text{rock fragments}}}}$$

$$\text{SOCstock}_i = \text{SOCcon}_{\text{fine soil}} \times BD_{\text{fine soil}} \times \text{depth}_i$$

## 2.9. Statistical analyses

The WSA distribution data and concentrations of bulk SOC and N were analysed by two-way ANOVA to detect effects of land management treatment, aggregate size category, and interactions between these variables. The data met all the test assumptions. For comparisons of the effects of land management treatments (arable, CAL, UAL, and hedge) on each aggregate size category, one-way ANOVAs were used. No significant differences were found between CAL and UAL sites in any of the analyses. Therefore, these two treatments were combined into a single 'ley' treatment. Where one-way ANOVAs were significant, they were followed by *post-hoc* Tukey multiple comparison tests, to determine the effects of land management on either the WSA size distribution or the OC and N content within them. Fields were not included as a factor, but used as replicates, as all fields were on the same soil type with similar texture (Hallam et al., 2020; Holden et al., 2019) and those included in the final analyses had comparable management histories.

## 3. Results

### 3.1. Water-stable aggregate distribution

A two-way ANOVA revealed a statistically significant interaction of aggregate size and land management (arable, ley, hedge) on the proportion of soil weight in each fraction ( $p < 0.001$ ). Three years after the arable soil had been converted to ley there was significantly lower proportions of soil in the  $<53 \mu\text{m}$ ,  $53\text{--}250 \mu\text{m}$  and  $250\text{--}1000 \mu\text{m}$  WSA fractions, but greater proportions within the  $1000\text{--}2000 \mu\text{m}$ , and especially, in the  $>2000 \mu\text{m}$  WSA fractions in ley soil compared to arable (Tukey test,  $p < 0.05$  in all cases; Fig. 3). The most striking difference was in the proportion of large

macroaggregates ( $>2000 \mu\text{m}$ ) accounting for 7.4 % of the arable soil but 39.4 % of the soil mass in the leys, a 5.3-fold difference (Fig. 3). The contribution of large macroaggregates in the ley were similar to that seen in the hedge soil (Fig. 3). When the two largest macroaggregate sizes are combined into a  $>1000 \mu\text{m}$  fraction, the proportion of soil mass in this fraction was larger in the ley soil at 48.7 %, compared to 12.3 % in the arable field (Tukey test,  $p = 0.006$ ). Similarly, the hedge soils contained much higher proportions of soil in the  $>1000 \mu\text{m}$  aggregate fraction than arable soil, at 50.9 % of the total soil weight (Tukey test,  $p = 0.009$ ). There were no significant differences between the proportions of soil in these macroaggregates in the hedge and ley soils (Tukey test,  $p = 0.96$ ).

### 3.2. Concentrations of organic carbon and nitrogen

With the exception of the  $>2000 \mu\text{m}$  WSA fraction for OC, there was a larger mean concentration of OC and N in all the aggregate size fractions in the ley compared to arable soil, evidenced by no interaction between aggregate size fraction and OC and N% in a two-way ANOVA ( $p = 0.99$ ), but these increases were not significant (Tukey test,  $p > 0.05$ ; Fig. 4a). There was also consistently higher OC and N% stored in hedge soils across all fractions compared to the arable and ley soils, however, this was only significantly higher than the arable soil in the  $1000\text{--}2000 \mu\text{m}$ , and the  $>2000 \mu\text{m}$  fractions, and in this latter case it was also significantly higher than in the leys (Tukey test,  $p < 0.05$ ). The similar pattern of responses in OC and N storage suggests a close coupling of OC and total N accumulation in aggregates (Fig. 4).

### 3.3. Ratios of organic carbon and nitrogen concentrations

The OC:N ratios showed modest variation between the WSA fractions in the soils under different management, ranging from 9.0 in the  $<53 \mu\text{m}$  aggregates in the arable soils to 11.7 in the  $250\text{--}1000 \mu\text{m}$  aggregates in the hedge soils (Fig. 5a). There was a significant interaction between aggregate size class and land management (two-way ANOVA,  $p = 0.006$ ). In the three smallest aggregate fractions ( $<1000 \mu\text{m}$ ) the OC:N ratios increased with increasing aggregate size, with hedge soils having significantly higher OC:N ratios than the arable and ley soils ( $p < 0.05$ ). In contrast, in the two largest aggregate fractions ( $>1000 \mu\text{m}$ ), the arable soils had the highest OC:N ratios, being significantly higher than that found in the ley soil in the  $1000\text{--}2000 \mu\text{m}$  fraction ( $p < 0.05$ ).

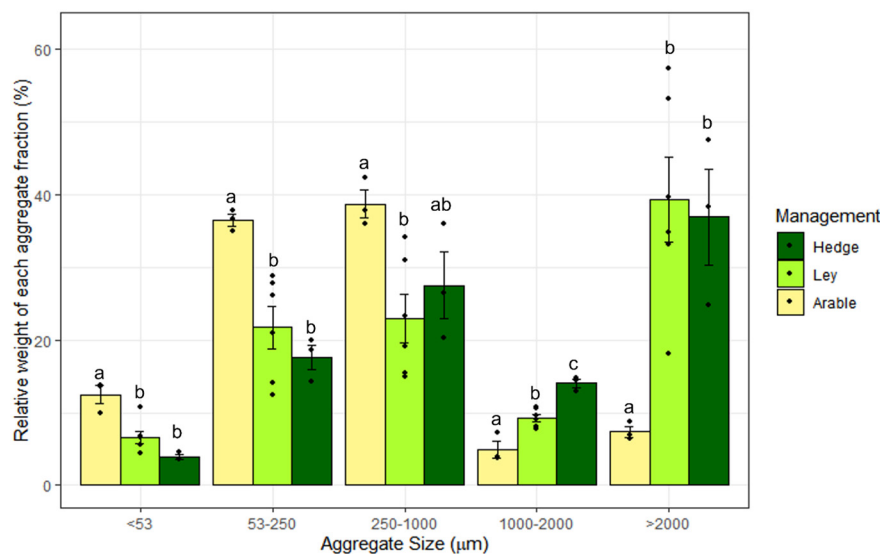
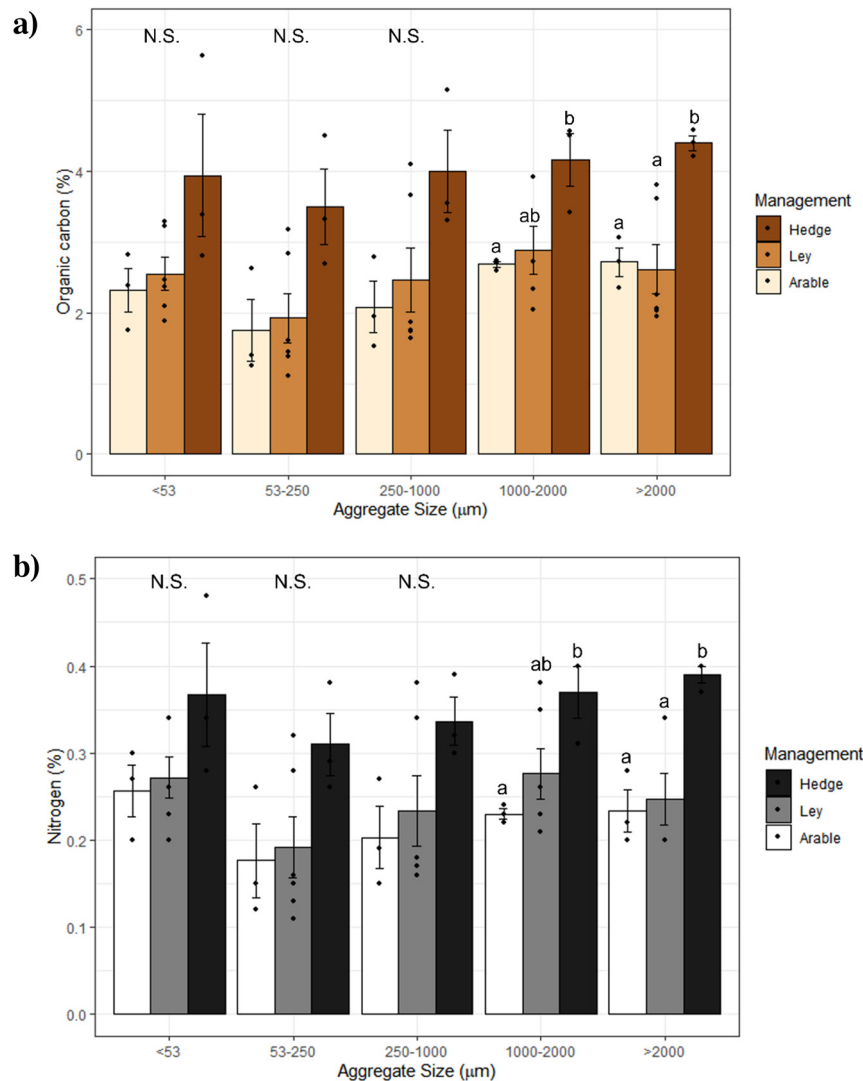


Fig. 3. The mean contribution of five different aggregate size fractions to soil mass under three different land managements (arable, three-year ley and hedge) with raw data points. Columns with different letters above them are significantly different from each other within the same aggregate size fraction (Tukey test,  $p < 0.05$ ). Error bars represent standard error.



**Fig. 4.** The (a) organic carbon % and (b) nitrogen % content of different WSA sizes for three land uses (arable, ley and hedge), showing raw data points. Bars with different letter codes above them are significantly different from each other within the same aggregate size fraction (Tukey test,  $p < 0.05$ ). Aggregate size groups with 'N.S.' above the bars means a non-significant one-way ANOVA result ( $p > 0.05$ ), therefore a post-hoc Tukey test was not performed. Error bars represent standard error.

Plotting OC against N content and analysis by linear regression showed strong evidence of co-accumulation of OC and N, reflected in the high  $R^2$  values (Fig. 5b). The slopes of the fitted regression lines (i.e., the OC:N ratios) systematically increased from arable (9.95) to ley (10.71) to hedge (13.66), with pairwise comparisons revealing no significant difference in the OC:N regression between arable and ley soils ( $p = 0.80$ ), but hedge soil having a steeper gradient than both arable ( $p = 0.03$ ) and ley soils ( $p = 0.02$ ).

### 3.4. Organic C and N distribution by aggregate fractions

Since the amounts of soil in each aggregate size fraction and the concentrations of OC and N within those fractions varied between the arable, ley and hedge soils, the proportions of soil mass by size fraction are presented in Fig. 6a to aid interpretation of these data. There were major differences in the composition of the different aggregate size fractions between the arable and ley soil, with significantly less soil in the three WSA fractions  $<1000 \mu\text{m}$  and significantly more in the two fractions  $>1000 \mu\text{m}$ , resulting in the ley being more structurally similar to the hedge than arable soils.

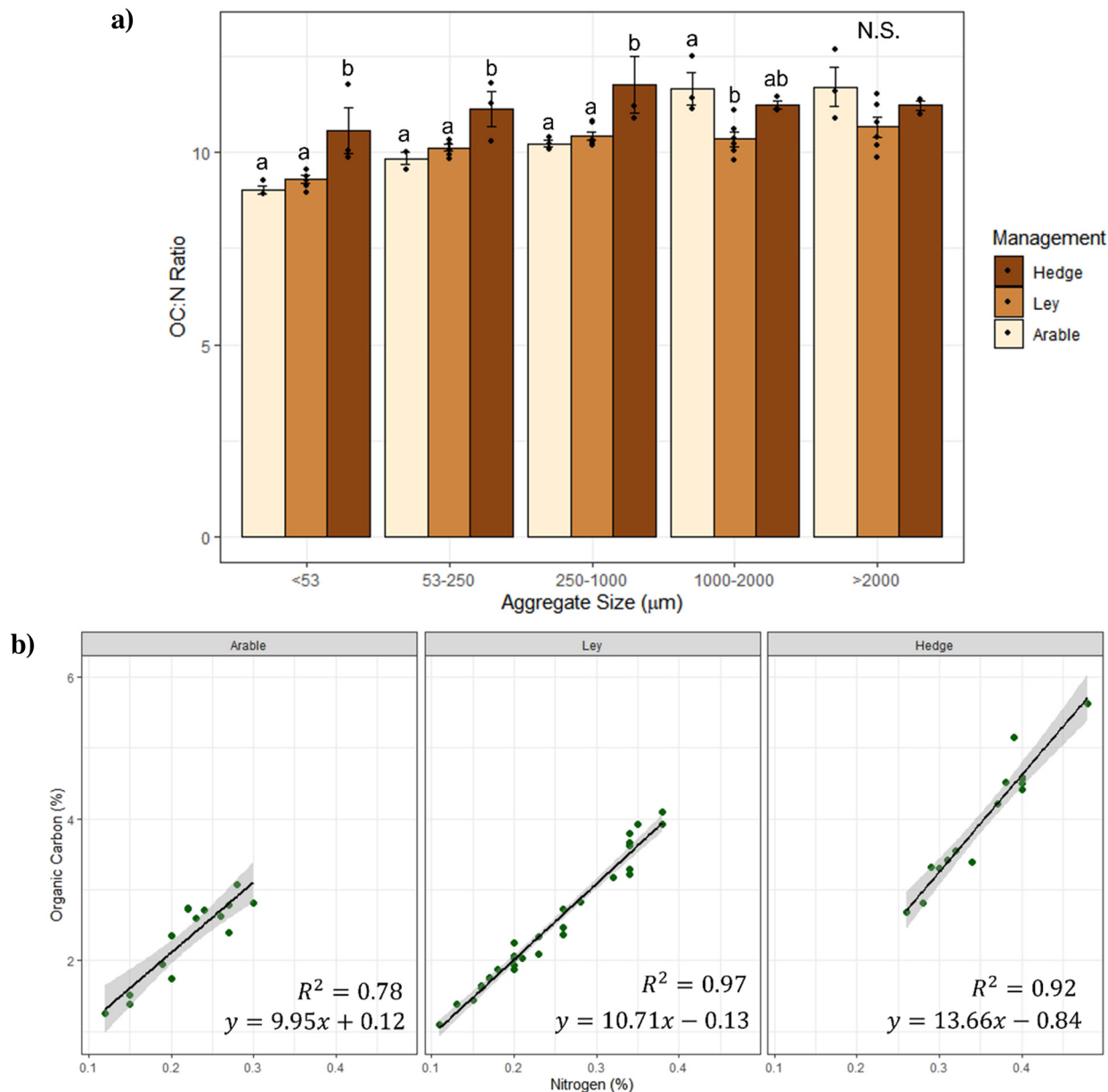
While a one-way ANOVA showed a significant effect of land management on bulk SOC content ( $p = 0.02$ ), there was no significant difference

in bulk SOC concentration between arable and ley soils (Fig. 6b, Tukey test  $p = 0.77$ ). The bulk SOC concentration in the ley soil was larger than that observed in the arable soil (2.43 % compared to 2.06 %, respectively), and both had significantly smaller concentrations than hedge soils (4.04 %) (Tukey test,  $p = 0.03$ ,  $p = 0.02$ , respectively; Fig. 6b). The ley did, however, have significantly (5.1-fold) greater OC stored within the  $>2000 \mu\text{m}$  fraction compared to the arable soil (Tukey test,  $p = 0.03$ ).

Land management also had a significant effect on bulk soil N content (ANOVA,  $p = 0.04$ ), but a post-hoc Tukey test revealed no differences between arable, ley or hedge soils (Fig. 6c;  $p > 0.05$ ). Total N (Fig. 6c) in the ley (0.23 %) and hedge soil (0.36 %) was greater compared to the arable soil (0.20 %). N storage in different WSA sizes (Fig. 6c) showed a similar pattern to that of OC, with the ley again having greater N% in the  $>2000 \mu\text{m}$  fraction (Tukey test,  $p = 0.03$ ), whereas significantly more N was stored in the smallest microaggregate fraction ( $<53 \mu\text{m}$ ) of the arable soil compared to after three years of ley (Tukey test,  $p = 0.03$ ).

The SOC stock in the leys was  $22.6 \text{ Mg ha}^{-1}$  compared to  $20.3 \text{ Mg ha}^{-1}$  in the arable fields and  $30.0 \text{ Mg ha}^{-1}$  under the hedges. This shows that the leys accumulated  $2.3 \text{ Mg C ha}^{-1}$  in the top 7 cm over the three years, which equates to an accumulation rate of  $0.77 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  ( $2.83 \text{ Mg CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ ). There has been criticism of studies comparing soil C stocks





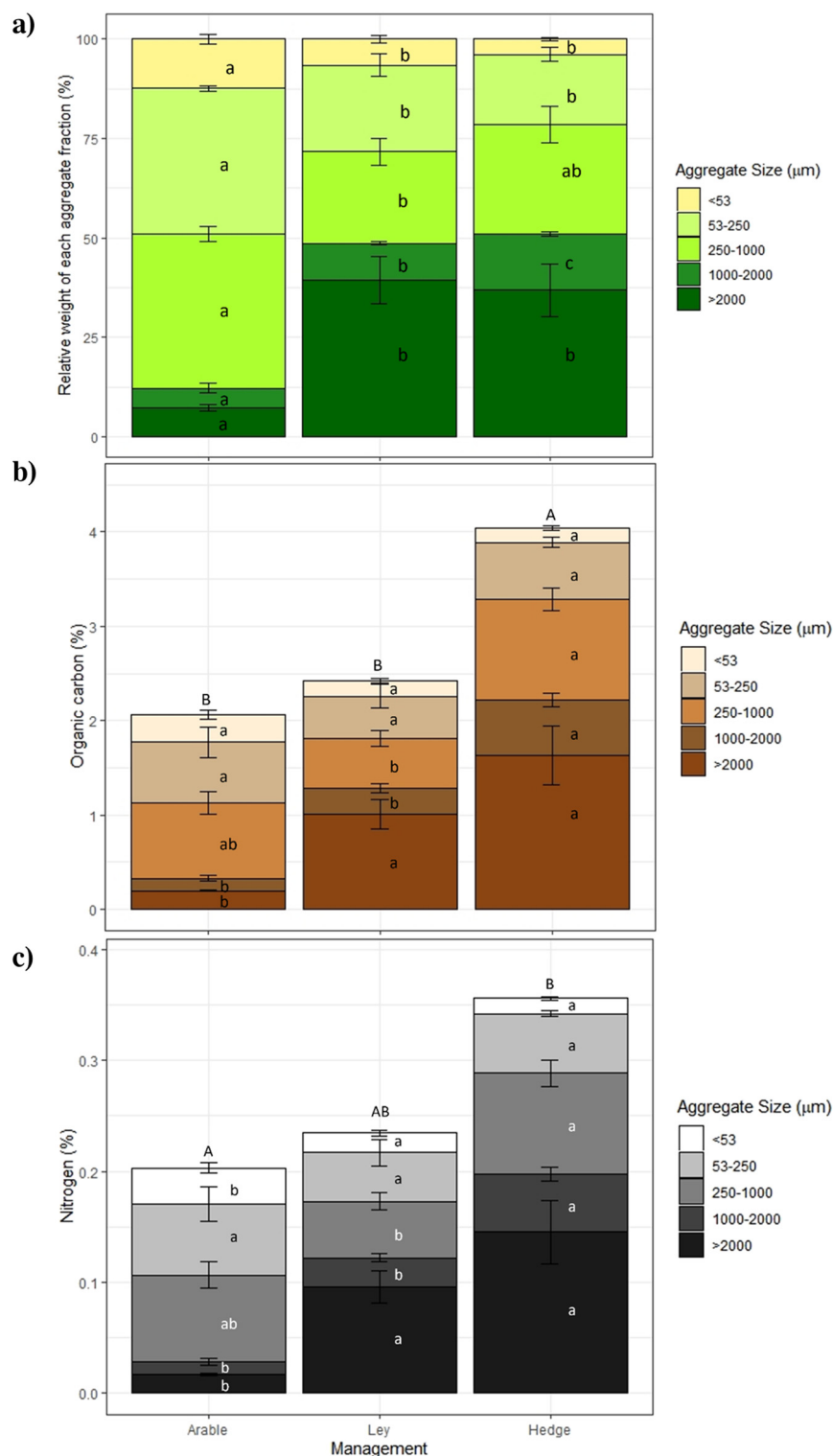
**Fig. 5.** (a) Bar chart and (b) linear regressions showing the effects of land management under permanent arable, three-year ley and hedgerow soils on the mean ratios of organic carbon: nitrogen storage, across five aggregate size fractions, with raw data points. Equations of the lines show their gradients, y-intercept, and the adjusted R-squared values. Grey bands either side of the regression line show the 95 % confidence.

in arable controls and fields under changed management without knowledge of baseline concentrations (Sanderman and Baldock, 2010). Of particular concern are situations where the baseline soil C concentrations are continuing to decrease from historical land use or management change. In this case, differences in soil C stocks between more recently imposed treatments measured at a single time point will not reflect net change in overall soil C storage, and if treatment differences are interpreted as C sequestration these would provide misleading overestimates. Our study has avoided this problem by having arable control fields that have C concentrations depleted to a very low baseline having been consistently ploughed, harrowed and annually cropped for >20 years. Prior to this, going back in total of 45 years to 1970, the fields only had a total of six years under leys, with cropping all the other years. This is corroborated by unpublished soil bulk density core sample data to 7 cm depth from the arable areas of the same fields in February 2013 (Marshall-Harries, 2013), five years before the present study, and two years before the leys

were sown where the OC stock to 7 cm depth was 17.2 Mg ha<sup>-1</sup> (standard error of  $\pm 0.5$ ,  $n = 3$  fields). These fields were sampled again in February 2017, one year before the present study, finding OC stocks to 7 cm depth at 18.0 Mg ha<sup>-1</sup> (standard error  $\pm 1.0$ ,  $n = 3$  fields), suggestive of a slight baseline improvement in SOC stocks, rather than any further declines. These differences were not statistically significant ( $p > 0.05$ ), and may be due to inherent soil variability, or possibly some slight recovery following the growing of potatoes in 2009 in our study fields, as conventional arable rotations with root crops were shown to deplete SOC (0.82 %) compared to cereals (0.94 %) in the 70-year study by Johnston et al. (2017).

The hedge soil value is the maximum benchmark value for this soil since an unpublished study comparing hedge and adjacent deciduous woodland in the same fields (Marshall-Harries, 2013) found no difference in SOC concentrations. Bulk soil N was also greater after three years of ley at 2.18 Mg ha<sup>-1</sup> compared to 2.0 Mg ha<sup>-1</sup> in the arable soil, with hedge soils storing the most at 2.6 Mg ha<sup>-1</sup>.





**Fig. 6.** The (a) soil aggregate size distribution by weight, (b) SOC and (c) N storage and where these are stored within the five aggregate size fractions. Aggregate fractions of the same size class with different lowercase letter codes denote significant differences between arable, ley or hedge means (Tukey test,  $p < 0.05$ ). Bars with different capital letter codes above them have significantly different total concentrations (Tukey test,  $p < 0.05$ ).

#### 4. Discussion

Our study corroborates and extends recent research demonstrating that the reintroduction of grass-clover leys into arable rotations for 19 months can result in remarkably large and rapid changes in soil structure (Berdini et al., 2021). The results now presented shows that three years of ley leads

to major reassembly of larger aggregates and this drives increases in OC accumulation into large macroaggregates and reductions in the proportion of soil mass in smaller aggregates. From the 'space for time' experimental approach, the differences seen between arable and ley soils in the same fields that were previously under long-term arable cropping are interpreted as changes in soil properties arising from a three-year arable-to-leys conversion.

#### 4.1. Bulk OC and N accumulation

The non-significant 11.3 % increase (from 20.3 to 22.6 Mg ha<sup>-1</sup>) in bulk SOC stock to 7 cm depth after the arable soil has been under ley for three years is consistent with results seen in other short-term ley and grassland studies. For example, Gosling et al. (2017) reported a non-significant 5 % increase in SOC% to 10 cm depth one to two years after introducing grassland into intensive arable cultivation and Puerta et al. (2018) found a non-significant increase of 8 % in SOC to 6 cm in a two year grass-clover ley following conventional arable cropping. Although not statistically significant, this increase in OC stock equates to an annual accumulation rate of 0.77 Mg OC ha<sup>-1</sup> in the top 7 cm of soil. This is towards the highest rates of soil C accumulation achieved through restorative land-use practices, including the adoption of conservation tillage and cover crops, which range from 0.05 to 1 Mg C ha<sup>-1</sup> (Lal, 2004a). However, it is well within the range of C accumulation rates found on conversion of arable land to grasslands reviewed by Conant et al. (2017), who found a mean of 0.87 Mg C ha<sup>-1</sup> yr<sup>-1</sup> across 93 studies globally. This was as part of a review of over 655 studies of effects of grasslands on SOC of which over 70 % only sampled soil up to 20 cm depth. While our shallow sampling, to only 7 cm depth, is likely to have underestimated the total SOC stock changes due to the leys, by far the most important changes in SOC due to grassland management have been found to occur near the surface, with subsoil on average contributing only half the increases in SOC stocks gained in the topsoil (Conant et al., 2017). Similarly, reassembly of soil macroaggregates in arable to grassland conversions starts at the surface and can take many years to effect significant changes below 10 cm depth as shown in Australia (Greacen, 1958).

Our comparison of ley bulk SOC against the arable field is based on the assumption that the permanent arable field have reached a relatively constant low value of SOC. This assumption is supported by previous studies finding similar SOC stocks year-to-year at the same SoilBioHedge field site (Marshall-Harries, 2013; Shaw, 2018). Previous research by Baveye et al. (2018) also showed that converting grassland to arable reduces SOC stocks fastest initially before reaching a new lower equilibrium, with the rate of loss progressively decreasing year on year. For example, in land that was already arable, the SOC stock remained at nearly 40 t ha<sup>-1</sup> for >40 years, indicating that land under long-term arable cultivation shows very little year-to-year variation in C content and bulk density. Research by Johnston et al. (2017) also shows over 70 years, %OC only declined from 0.98 to 0.94 in an all-arable rotation with mainly cereals, through a period of intensification of agricultural practices. This small decline over a long time reinforces the view that after several decades of continual arable cropping SOC% will be close to equilibrium value.

#### 4.2. OC and N in soil water-stable aggregates

Although we see no significant changes in bulk OC stock from arable-to-ley conversion, there are large and highly significant increases in the amounts of SOC in the >2000 µm fraction following arable to ley conversion (Fig. 6b). This is consistent with soil macroaggregation being strongly linked to soil C accumulation in mineral soils, where OC bound to microaggregates is further protected within macroaggregates (Aoyama et al., 1999; Mikha and Rice, 2004; Puget et al., 2000; Yu et al., 2015; Zhou and Pan, 2007). The rapid reassembly of WSA >2000 µm after the three years of ley, with recovery to proportions seen under hedges, was a surprisingly fast and clear indicator of substantial improvement in soil quality. Previous studies of OC accumulation in agricultural soils under different management treatments and OM inputs have suggested that OC saturates in macroaggregates so that the proportion of the soil volume that consists of macroaggregates controls the capacity of soil to store C (Yu et al., 2015). In our study, while the proportion of soil volume in macroaggregates recovered to the proportions seen in hedge soils, the concentration of OC in these aggregates was far from saturation, but the capacity for this additional C storage had been generated in three years. Meanwhile, the consistency of aggregates in the 250–1000 µm size fraction (Fig. 3) suggests that in this

soil system, binding agents for this fraction are more permanent. This is a little different from that described in the paradigm by Tisdall and Oades (1982), which suggests that more permanent aggregating agents are involved in the 53–250 µm class.

The preferential storage of OC within large macroaggregates has previously been seen in soils under no-till management compared to conventional tillage (Messiga et al., 2011; Wright and Hons, 2005). Li et al. (2016) also found that soils with enhanced soil aggregation rates stored more C within macroaggregates compared to microaggregates. The lack of soil disturbance in the ley is known to allow for a slower rate of macroaggregate turnover, the formation of new microaggregates within macroaggregates and with them, the accumulation and stabilization of mineral-associated OC (Six et al., 2002, 2000, 1999).

With more SOC stored within large macroaggregates, more OC is protected from microbial decomposition (Balesdent et al., 2000; Plante and McGill, 2002). The entrapment of microaggregates within macroaggregates provides physical protection (Bronick and Lal, 2005; Devine et al., 2014), with external layers being built upon older C stored in the aggregate interior (Santos et al., 1997). If SOC is more protected from microbial decomposition, it is more likely to be stored over a longer period, resulting in a net C sequestration into the soil rather than loss to the atmosphere as CO<sub>2</sub> (Lal, 2004b; Stavi and Lal, 2013). In contrast, the disaggregated, frequently cropped and tilled arable soil leaves OC more available for microbial decomposition and prone to erosion (Graves et al., 2015) via overland flow, which preferentially moves the lighter soil particles rich in OC, including microaggregates.

The leys are highly effective components of a regenerative agricultural system causing rapid reassembly of large macroaggregates which must be attributable to the combination of land management practices that change on arable to ley conversion. These include cessation of arable cropping and tillage, resulting in minimal soil disturbance and an increase in OM inputs predominantly from the roots, which show greater recalcitrance than shoot litter (Rasse et al., 2005) as apart from in the first year of the leys, grass clippings were removed after mowing. Mowing and mulching of grass and clover has been employed in a number of previous studies on soil fertility building in arable rotations (Deguchi et al., 2014; Moyo et al., 2015, 2016), but may offer limited benefits over mowing and removal for soil fertility depending on the extent to which shading by the mown material delays regrowth, and without nitrogen-offtake, clover may decline in favour of grass species, reducing the rate of soil N inputs via symbiotic fixation. Because there is no immediate financial return from mowing and mulching, silage or hay production is more likely to be used in stockless systems, but will tend to give lower inputs of C and N to the soil than where livestock are reintegrated into the ley phase of an arable rotation, recycling N and C in their urine and dung.

The ceasing of tillage in the ley would enable roots and hyphae to hold macroaggregates together for longer (Portella et al., 2012). Studies conducted in the same fields as the present study have found rapid recovery of biological agents of soil aggregation in the leys including earthworm populations (Prendergast-Miller et al., 2021) which were shown by experimentation to be important agents involved in regenerating soil structure (Hallam et al., 2020). We would also expect substantial increases in the extent and persistence of mycorrhizal mycelial networks that aid soil aggregation and soil carbon accumulation (Wilson et al., 2009) in the evergreen leys given the adverse effects of tillage on mycorrhizal activities in arable fields (Garcia et al., 2007).

The beneficial effects of the leys on biological aggregating agents are also likely to be facilitated by the pause in agrochemical use while the land is not in cultivation, as the herbicide glyphosate used in the arable parts of the field can adversely affect mycorrhiza (Caesar-TonThat et al., 2010; Druille et al., 2013a, 2013b; Helander et al., 2018). Similarly, the Clothianidin seed coating used on the cereals that were sown in these fields is the most toxic widely used neonicotinoid to earthworms (Yang et al., 2017). While it is extremely difficult to assess effects of the complex mixtures of about 20 different agrochemicals that are applied to grow arable crops like wheat on soil organisms, soil structure and carbon storage,

a first estimate of the effects of such mixtures on earthworms has been reported by [Maggie and Tang \(2021\)](#). This suggests earthworm population numbers may be reduced by about 5 % by agrochemical residues, which is a modest effect compared to effects of tillage ([Edwards and Lofty, 1982](#)), and introducing organic rotations with grazed legume-rich leys, where earthworm populations increase by over 80 % compared to conventional arable cropping ([Riley et al., 2008](#)).

The increase in soil N stocks in the leys through biological N fixation by clover will reduce N fertiliser application for subsequent cereal crops ([McKenna et al., 2018](#)). As the production and use of N fertiliser accounts for 43 % of the global warming potential in the lifecycle analysis of a loaf of bread ([Goucher et al., 2017](#)), this ley N fixation has the potential to reduce net emissions from cereal growing. The 0.18 Mg ha<sup>-1</sup> increase in total N seen after arable soil was put under ley for three years equates to 180 kg ha<sup>-1</sup> (or 60 kg ha<sup>-1</sup> yr<sup>-1</sup>), which is greater than the average yearly N application rates of manufactured fertiliser in England, at 143 kg N ha<sup>-1</sup> for 2019/20 ([Defra, 2021a](#)). Although, it is important to note that fertiliser application is inorganic N whilst the majority of the accumulation under ley will be organic N. Preferential N storage into large macroaggregates, which are less easily washed away by heavy rainfall events, could also result in reduced nitrate leaching into groundwater, as shown in previous studies from the introduction of grasslands into arable crop rotations ([Kunrath et al., 2015](#)).

#### 4.3. OC:N ratios

In this study, we see significant changes in the OC:N ratio between land management treatments, with the hedge soil having a higher OC:N ratio in aggregates <1000 µm and ley soil having a lower OC:N ratio compared to the permanent arable soil in the 1000–2000 µm fraction ([Fig. 5a](#)). This was unexpected, as [Holden et al. \(2019\)](#) and [Berdeni et al. \(2021\)](#) both found no significant effect of land management on C:N ratios at the same study site. [Edmondson et al. \(2014\)](#) also reported no change in soil C:N between arable and pasture, which maintains a similar plant community to leys. The increasing OC:N ratio along the arable-ley-hedge gradient suggests that with increased OC storage there is a progressive shift towards less nitrogen being stored for each unit of OC. This may also reflect a gradient of decreasing N inputs from highly nitrogen fertilized arable field, to unfertilized ley with nitrogen-fixing legumes to legume-free, unfertilized hedges.

#### 4.4. Comparison of field to hedge soils

Hedges comprise the most common field boundaries in lowland arable landscapes in the UK, and in much of Europe ([Holden et al., 2019](#)). Consequently, soil quality benchmarking in fields against hedgerow soils is particularly useful for evaluating the effects of short- to medium-term management changes on the extent to which agricultural soil properties are recovering towards their local potentials for the particular soil type, landscape, and climatic environment. In many lowland arable landscapes, there are few permanent grasslands to serve as benchmarks for C stock changes, therefore hedgerows provide a more ubiquitous alternative. Our approach in benchmarking linked changes in macroaggregation and in C and N accumulation in arable-to-ley conversion against the soil properties in the hedges at the field margins provides additional insights and context to the effectiveness of the leys in soil quality restoration and fertility-building. SOC stocks often change with land management change, but the rate of change progressively decreases towards a new equilibrium for a particular management, soil type, and climate context such as in arable to grassland conversions ([Baveye et al., 2018](#)). An earlier unpublished study conducted at the same farm found that soils under the arable field hedges had the same WSA size distributions as soil from the adjacent mature mixed species deciduous woodlands ([Marshall-Harries, 2013](#)), supporting the use of hedge soil as an “end point” benchmark of soil macroaggregation. However, the woodland soils contained about 16 % more OC in the top 0–7 cm, although this may be due to a lower stone content, as tilling of the arable fields brings up the shallow limestone bedrock at this site,

some of which has been deposited at the edge of the fields and under hedgerows.

#### 4.5. Transition to the incorporation of leys into arable rotations

SOC is lost at a much greater rate after returning to arable from grassland than it can be built up from the introduction of grassland ([Jensen et al., 2020](#)). Therefore, how soil is managed in between leys is key in maintaining some of their benefits through full cycles of arable rotations. Tillage can cause a sharp decline in the large WSAs ([Low, 1972](#)), the breakdown of which would be paired with the loss of the OC and N they contain, easily reversing the benefits provided by the ley. Ideally, the inclusion of a ley into arable rotations needs to be carried out alongside other regenerative agriculture practices such as direct drilling, or reduced tillage to preserve more of the regenerative effects of the leys on soil health ([Chan and Mead, 1988](#); [Puerta et al., 2018](#)), and this may deliver co-benefits of reduced residual weed germination from light-requiring seeds compared to tillage, and can be managed effectively alongside crop rotation ([MacLaren et al., 2021](#)). In arable-to-ley rotations using conventional ploughing and intensive annual crop production, soil C stocks initially increase compared to permanent arable cropping, but then oscillate around a higher long-term mean, as shown by [Johnston et al. \(2017\)](#). They found over 70 years that a three-year grass-clover ley in a five-year rotation with conventionally grown arable crops increased SOC concentrations from 0.98 % in permanent arable land to 1.23 % after 28 years, apparently approaching a new equilibrium which was estimated to be about 1.3 % C. Depletion of SOC gains by leys is an inevitable consequence of ploughing and cropping ([Low, 1972](#); [Poulton et al., 2018](#)). However, the extent to which such losses can be delayed or prevented remains unknown for the new situation where leys may be introduced as a regenerative agriculture method to assist transitioning to no-till or minimal tillage, and use of legume under sowing and cover crops in rotations. The longer-term effects of leys in such management systems has recently been highlighted by [Cooledge et al. \(2022\)](#) as an urgent research priority.

Inclusion of leys into crop rotations needs to be financially sustainable and be economically favourable over continual cropping with full rotations to be successful. Some financial returns can be gained by grazing leys or in stockless systems, through production and selling of silage and other forage products together with fiscal incentives via government managed agricultural subsidies, which has shown to be successful in Sweden ([Poeplau et al., 2015](#)). The introduction of the Environmental Land Management (ELM) scheme in England to help achieve Defra's (the UK government's Department for Environment, Food and Rural Affairs) 25 Year Environment Plan goals of net zero C emissions, with the stated goal that all of England's soils are to be sustainably managed by 2030, is supporting actions to reward farmers to encourage C-friendly farming through better soil management ([Defra, 2021b](#)), and has identified leys as one component of this strategy.

#### 4.6. Application of methodology for soil quality monitoring

From our findings, and previous related work, we conclude that the measurement of OC within large macroaggregates compared to bulk SOC is a robust early indicator of soil quality improvement, as this enables detection of statistically significant and functionally important short-term changes in soil structure and quality. While there was no significant difference in bulk SOC stocks between arable and ley soils, we were able to detect large and significant differences in the amount of OC stored within the >2000 µm WSAs ([Fig. 6](#)). Water-stable macroaggregates are key functional indicators of good soil structure, being associated with reduced bulk density, increased macroporosity, and physical protection of OC against microbial decomposition ([Balesdent et al., 2000](#); [Plante and McGill, 2002](#)). Observations of preferential storage of OC and N in the largest aggregates compared to the bulk soil is a clear indicator that the soil biology, chemistry and structure are regenerating, in the present study, under leys. Wider application of this methodology to assess WSA size distribution and the OC and N% within large macroaggregates would be helpful to guide

farmers as to the effectiveness of management changes for soil health. The methodology could be integrated into national monitoring of soil health at field-to-landscape scales, for example in England's ELM scheme, which is currently developing an indicator framework for assessing improvements in public goods provided on farmland (Defra, 2021b).

Although we measured the distribution of five aggregate size fractions and the OC% and N% within each of them, the key fraction of interest was the largest. Therefore, for more economical and higher through-put processing, it may be appropriate to collect only the >2000 µm wet-sieved fraction and the remaining soil fraction smaller than this and analysing these two fractions for OC% and N%. This will give information on the proportion of large soil macroaggregates and how much OC and N are stored within them compared to bulk SOC and N, which are already commonly measured. Further studies are required to test this approach on a wider range of soil textures and types, which can affect macroaggregation processes (Denef et al., 2004; Denef and Six, 2005; Rakhsh et al., 2017), and so determine if the two-fraction (>2000 µm and < 2000 µm) approach would be applicable to the main types of mineral soils used in arable farming. This methodology could potentially help not only determine long-term trends in overall bulk SOC and N, but also detect shorter-term improvements in soil structure and functions which are not picked up from bulk SOC measurements alone.

## 5. Conclusion

Our findings affirm the importance of including grass-clover leys in crop rotations to improve soil structure through increasing the prevalence of large macroaggregates. There was no significant difference in bulk SOC and N% or SOC and N stock after the arable fields are under ley for three years. However, this equated to an average annual SOC accumulation rate of 0.77 Mg OC ha<sup>-1</sup> in the top 7 cm of soil. Compared with adjacent arable soil, we detected a substantially greater OC and N storage within large macroaggregates, structures which are known to provide better protection for long-term C storage, with an increase of 7.6 Mg ha<sup>-1</sup> and 0.18 Mg ha<sup>-1</sup> macroaggregate-bound OC and N, respectively, in the arable-to-ley conversions after three years. We propose that monitoring OC and N changes in large macroaggregates is a sensitive and useful early indicator of changing soil quality and structure that potentially could be applied in routine soil health monitoring, and for which farmers should be rewarded, but this needs to be tested on a wider range of arable soil types. Further research needs to focus on which other land management practices improve these traits, and whether by only measuring two fractions, the >2000 µm and < 2000 µm WSA, and benchmarking changes relative to undisturbed hedgerow soils would provide an efficient way of routinely monitoring changes in soil functioning in response to land use or management changes.

## CRediT authorship contribution statement

**Emily J. Guest:** Investigation, Writing – original draft, Formal analysis, Project administration. **Lucy J. Palfreeman:** Investigation, Writing – review & editing. **Joseph Holden:** Funding acquisition, Writing – review & editing. **Pippa J. Chapman:** Funding acquisition, Writing – review & editing. **Les G. Firbank:** Funding acquisition, Writing – review & editing. **Martin G. Lappage:** Investigation, Project administration, Resources. **Thorunn Helgason:** Funding acquisition, Writing – review & editing, Supervision. **Jonathan R. Leake:** Conceptualization, Funding acquisition, Writing – original draft, Supervision.

## Data availability

Data will be made available on request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The field design sampled for use in this study was part of the NERC Soil Security Programme funded project 'SoilBioHedge' (NE/M017044/1, NE/M017095/1, NE/M017079/1). Emily Guest's PhD is funded by the White Rose BBSRC DTP in Mechanistic Biology (BB/M011151/1). Lucy Palfreeman was funded by the Sheffield Undergraduate Research Experience (SURE) scheme funded by the BBSRC Research Experience Placement Scheme. We are thankful for Irene Johnson, Dave Johnson, Anthony Turner and Roscoe Blevins for their help in the laboratory and Hetty Shaw for the use of her bulk density measurements presented in her master's thesis work.

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