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Research article

The potential to increase grassland soil C stocks by extending reseeding intervals is dependent on soil texture and depth

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ABSTRACT

Grasslands account for ~30% of global terrestrial carbon (C), of which most is stored in soils and provide important ecosystem services including livestock and forage production. Reseeding of temporary grasslands on a 5-year cycle is a common management practice to rejuvenate sward productivity and reduce soil compaction, but is physically disruptive and may reduce soil organic carbon (SOC) stocks. However, research to date is limited, which impacts on the ability to optimise grassland management for climate change mitigation. To determine whether extending the time interval up to 20 years between grassland reseeding can increase stable SOC stocks, a soil survey was conducted across three UK grassland chrono-sequences comprising 24 fields on contrasting soil types. We found that grassland SOC stocks (39.8–114.8 Mg C ha^{-1}) were higher than co-located fields in arable rotations (29.3–83.2 Mg C ha^{-1}) and the relationship with grassland age followed a curvilinear relationship with rapid SOC stock accumulation in the year following reseeding (2.69–18.3 Mg C ha^{-1} yr^{-1}) followed by progressively slower SOC accumulation up to 20 years. Contrary to expectation, all grasslands had similar soil bulk densities and sward composition questioning the need for traditional 5-year reseeding cycles. Fractionation of soils into stable mineral associated fractions revealed that coarse textured grassland topsoils (0–15 cm) were near-saturated in C irrespective of grassland age whilst loam soils reached saturation ~10 years after reseeding. Fine-textured topsoils and subsoils (15–30 cm) of all textures were under saturated and thus appear to hold the most potential to accrue additional stable C. However, the lack of a relationship between C saturation deficit and grassland age in subsoils suggests that more innovative management to promote SOC redistribution to depth, such as a switch to diverse leys or full inversion tillage may be required to maximise subsoil SOC stocks. Taken together our findings suggest that extending the time between grassland reseeding could temporarily increase SOC stocks without compromising sward composition or soil structure. However, detailed monitoring of the trade-offs with grassland productivity are required. Fine textured soils and subsoils (15–30 cm) have the greatest potential to accrue additional stable C due to under saturation of fine mineral pools.

1. Introduction

Agriculture and associated land-use change is a major contributor to atmospheric greenhouse gases (GHG's) with 10–14% of anthropogenic emissions directly attributable to agricultural production such as soil and livestock management (Paustian et al., 2016). Soils are the largest source of GHG emissions within the agricultural sector but improvements to soil management and agricultural intensification can reduce trace gas emissions (CH_4 and N_2O) and also sequester additional plant derived C as soil organic matter (SOM) (Burney et al., 2010; Smith et al., 2008). For example, increasing agricultural soil organic C (SOC) stocks in the top 30 cm by 0.4% (4 per mille) could temporarily compensate for the entirety of annual global emissions from anthropogenic sources (Minasny et al., 2017; Paustian et al., 2016). Therefore, targeting land management that increases SOC stocks has been recognized as a feasible strategy for achieving short to medium term land-based negative

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Table 1
Summary of sampling locations, climatic variables and predominant soil types. Mean minimum and maximum annual temperature and mean annual rainfall were taken from the nearest geographical met office weather stations and soil types were determined from the British Geological Survey UK Soil Observatory (UKSO) map viewer.

Site	Location	Number of fields sampled	Age Range (years)	Land Use	Mean maximum annual temperature (°C)	Mean minimum annual temperature (°C)	Mean annual rainfall (mm)	Elevation (m a.s.l.)	WRB Soil Type	Soil texture
Buxton	53°20′ N, 1°80′ W	9	0 to 15	Dairy Pasture/ Arable	11.9	5.3	1344	299–329	Cambisols	Silty Loam
Buscot Wick	51°68′ N, 1°68′ W	8	0 to 20	Dairy Pasture/ Arable	14.6	6.6	706	70–75	Cambisols and Gleysols	Loam
Bisterne	50°82′ N, 1°78′ W	7	0 to 9	Dairy Pasture/ Arable	15.1	6.3	877	13–17	Luvisols	Sandy Loam to Loamy Sand

emissions (Smith et al., 2008).

Grasslands are of major agronomic importance and are used primarily to produce forage for livestock grazing (Power, 2010; Smit et al., 2008). Grassland soils are also important global stores of C containing ~30% of all terrestrial soil C and have the potential to store more C than equivalent arable soils (Conant et al., 2001; Eze et al., 2018; Lal et al., 2018; Lavelle et al., 2012; Smith, 2014). Although the amount of C stored in grassland soils is locally controlled by soil texture and climate, SOC stocks can also be modified by land management (Jones et al., 2006). Management shown to influence SOC stocks ranges from variation in grazing intensity and frequency, the magnitude of fertilizer application, liming, sward species composition and the degree of physical disturbance to soils during farm operations (Conant et al., 2017; Eze et al., 2018; Ward et al., 2016).

In the UK, grasslands are the dominant land-use with more than half of agricultural land area classified as grassland (Perkins et al., 2000). Therefore, targeting grassland management that promotes soil C storage and reduces GHG emissions could play a major role in reducing emissions from the agricultural sector, which is currently responsible for 11% of UK GHG emissions (BEIS, 2022). Currently, however the majority of UK grassland management is strongly focused on maximising productivity and forage quality; allowing for high livestock stocking densities. Thus, the most commonly grown grass species are high yielding varieties such as *Lolium perenne* (perennial ryegrass) often mixed with *Trifolium* spp. (clover). Perennial ryegrass leys are highly nutritious, high yielding and remain the cornerstone of intensive grazing systems. However, these monoculture leys may only reliably last for between three and five years before substantial encroachment of unsown species, which can reduce productivity and forage quality (Defra, 2018; Velthof et al., 2010). Therefore, periodic reseeding is considered an important aspect of grassland management to maintain productivity. In the UK reseeding is most often performed every 3–5 years (Defra, 2018) and typically involves spraying off the existing sward with herbicide, soil tillage to prepare a new seedbed followed by sowing (Velthof et al., 2010). Whilst considered important for maximising grassland productivity, the associated physical disruption of soils can release substantial quantities of SOC (Linsler et al., 2013; Necpálová et al., 2014). Further, the spraying of grasslands with herbicides may also promote NO_3^- leaching and prime soil microbial denitrification processes, leading to an increased GHG management intensity due to N_2O emission (Buchen et al., 2017; Merbold et al., 2014; Seidel et al., 2009). 10% of UK grasslands are classified as rotational leys, which are under 5 years in age (Conijn et al., 2002). In any one year, approximately 20% of these leys would be ploughed and reseeded, amounting to about 300,000 ha ploughed grassland per annum (Conijn et al., 2002). Therefore, mitigating SOC losses by extending the interval between physically disruptive reseeding events could enhance grassland SOC stocks and be an effective climate change mitigation strategy. However, robust soil surveys of grassland chronosequences are required to establish how SOC stocks are related to reseeding intervals.

The current evidence as to whether increasing the time interval between reseeding events on SOC stocks is equivocal. Previous studies have either shown positive associations between bulk SOC stocks and grassland age (Iepema et al., 2022; Necpálová et al., 2014), transient relationship and C redistribution within the soil profile or no effect (Carolan and Fornara, 2016; Linsler et al., 2013). Although soil CO_2 fluxes are temporarily stimulated following cultivation and reseeding events (Drewer et al., 2017; Willems et al., 2011), the main mechanism of soil C loss appears to be the temporary reduction in gross primary production (GPP) rather than elevated soil respiration. Reseeding events are also performed relatively infrequently and C losses appear to be short-lived as grasslands can revert to C sinks after just one year (Schils et al., 2005). Moreover, recovery from short-term C stock declines following tillage and reseeding may take just 5 years (Linsler et al., 2013); the time at which tillage and reseeding often occurs for UK grasslands. Few studies have examined the distribution of grassland SOC into functionally distinct soil pools. This is important as it likely informs on the degree to which SOC stocks are vulnerable to physical disturbance during reseeding. The inconsistent relationship between time since reseeding and grassland SOC stocks may thus be influenced by the proportion of SOC stored in persistent soil pools with long residence times and vulnerable pools with short residence times. Particulate organic matter (POM) is largely made up of relatively undecomposed, lightweight fragments of organic material and has short residence times in soil whilst mineral associated organic matter (MAOM) is thought to be more persistent due to protection from microbial decomposition through association with soil minerals (Lavallee et al., 2020). The distribution of C between these functionally distinct pools in relation to grassland age has not been considered as a driver of the soil C response to tillage and reseeding. This is also important as it likely informs on the stability of SOC stocks under future management disturbance.

The potential to enhance stable SOC stocks by extending intervals between reseeding events is, to a large degree, dependent on the maximum SOC levels attainable in mineral associated soil C pools. and particularly SOC associated with fine particles (<20 μm) that is thought to be most stable (Hassink, 1997). There is empirical evidence to suggest that there exists an upper protective limit (saturation point) for the mineral associated C pool (Six et al., 2002; Stewart et al., 2007). Therefore, potential SOC sequestration or the “saturation deficit” may be estimated by subtracting current mineral stabilised C from estimated maximum mineral stabilised C (Angers et al., 2011; Wenzel et al., 2022). Maximum mineral stabilised C is often estimated using Hassink’s equation ($y = 0.37x + 4.07$) derived from least squares regression of measured fine fraction C against the mass proportion of fine soil particles in permanent grasslands (Hassink, 1997). However, this equation is not applicable across soils of varying mineralogies (Six et al., 2002), often underestimates the upper limit of SOC stabilization (Feng et al., 2013) and may not be applicable to UK grasslands (Paterson et al., 2021). Quantile regression provides more robust estimates of SOC sequestration potential and has recently been applied across a wide range of UK soils (Paterson et al., 2021).

Using a grassland chrono-sequence approach our study aimed to test whether grassland SOC stocks increased with time since reseeding across soils of varying texture and whether C accumulated in transient (POM) or persistent (MAOM) C pools. We hypothesized that SOC stocks (0–30 cm) would be locally controlled by soil texture but increase with grassland age (1); older grasslands would have a greater proportion of total SOC stabilised as MAOM across soils (2) and that the C saturation deficit of all soils would decrease with grassland age (3).

2. Material and methods

2.1. Site description and field selection

The study was conducted between November 2020 and February 2021 across three commercial dairy farms in England located on different soil types (Table 1). At each farm a chrono-sequence of between 7 and 9 temporary grass leys were selected based on age (years since reseeding), soil maps (UK Soils Observatory, BGS) (to minimize variation in soil type) and availability of comprehensive management history (Table 1, Supplementary Information: Table S1). Selection criteria of grassland fields were: used for grazing of livestock or grass silage or hay cutting; fertilized with >100 kg N ha^{-1} as inorganic fertilizer or slurry; sward composition consisting primarily of perennial rye grass (PRG) (*Lolium perenne*) and previous land use as arable or grassland. These criteria were applied to minimize differences between grasslands other than the time since reseeding. At each farm, a field currently in a winter wheat arable rotation was sampled to establish a baseline SOC stock at the presumed lowest point in a typical ley-arable-ley rotation. Grassland age and management history was provided by participating farmers.

2.2. Soil sampling and analysis

The surface soil (0–30 cm) was sampled from each field using a split-tube soil sampler (Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands) with an inner diameter of 4.8 cm to a depth of 30 cm. Ten cores were sampled within each field using a W pattern to capture within field spatial variability. A buffer of 20 m was used around the field margins to minimize potential edge effects and zones of compaction (entrances to fields and turning headlands) were avoided. Soil cores were divided in the field into 0–15 cm and 15–30 cm (measuring from the base of the core), individually bagged and returned to the laboratory in a cool box. The fresh mass of the 0–15 cm and 15–30 cm core sections was recorded and then homogenised by hand prior to subsampling for different analyses. A 20–30 g fresh subsample was taken for determination of pH. Another 20–30 g subsample was archived as a frozen sample (−20 °C). The remaining soil was set aside for determination of soil C and bulk density, together with any large stones and coarse roots (>5 mm) hand-sorted from the rest of the core. For bulk density and soil C determination, the fresh soil mass was recorded, and samples were then air-dried at 30 °C in a drying room until constant weight. Air-dried samples were re-weighed, sieved to 2 mm through a stainless-steel sieve and the mass and volume (measured using water displacement) of stones and roots remaining on the sieve were recorded. A subsample of the sieved, air-dried soil (20 g) was oven-dried (105 °C for 24 h) and moisture loss was recorded. The oven-dried subsample of soil was ground in a ball mill (Fritsch Planetary Mill) and a 100 mg subsample was used for the assessment of total C and N concentration using an elemental analyser (Leco Truspec Micro, Michigan, USA). Inorganic C was determined gravimetrically using the method described by Rodriguez (2016) and total organic carbon was determined by subtraction of any inorganic C from total C.

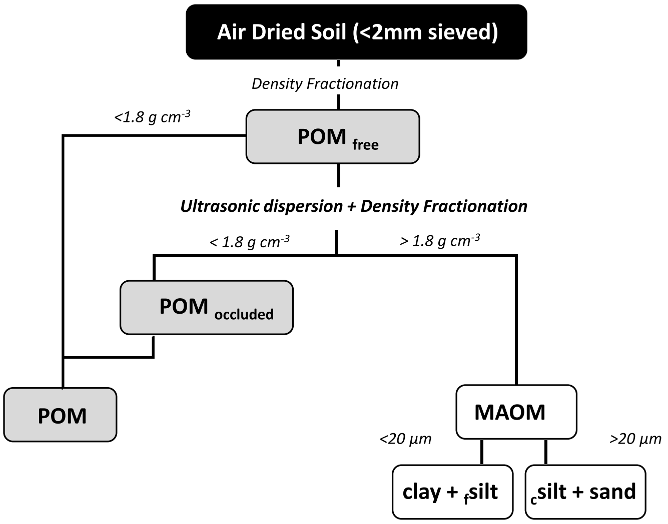

Fig. 1. Schematic of the fractionation procedure. Key: POM_{free} = non-occluded particulate organic matter, $\text{POM}_{\text{occluded}}$ = occluded particulate organic matter, MAOM = mineral associated organic matter, clay + ${}_{f}$silt = MAOM <20 μm, ${}_{c}$silt +sand = MAOM >20μm.

Soil pH was measured on fresh soil subsamples. These were sieved to 4 mm and then 10 g soil was mixed into a slurry with 25 ml deionized water and left to stand for 30 min. The pH of the suspension was then measured using a calibrated pH probe (Hanna pH210 Meter, Hanna Instruments Ltd, Bedfordshire, UK). Soil texture was analysed on sieved (<2 mm), air-dried soils by laser diffraction using a Beckman Coulter LS 13,320 analyzer (Beckman Coulter, Indianapolis, USA). Organic matter was removed prior to particle size analysis by digesting soils using hydrogen peroxide (Mikutta et al., 2005).

2.3. Soil C fractionation

Soil C fractionation was conducted on samples bulked per field at 0–15 cm and 15–30 cm using a physicochemical density fractionation method based on the procedure of Schrumpf et al. (2013), which is based on ideas from Golchin et al. (1994). Fig. 1 demonstrates the fractionation procedure. We distinguished between free particulate organic matter (POM) and occluded POM, which were combined to form the POM fraction and mineral associated organic matter (MAOM). MAOM was further physically separated by size into coarse (>20 μm) and fine (<20 μm) mineral fractions. 5 g subsamples of sieved, air-dried soil were placed in 50 ml centrifuge tubes with 40 ml Sodium Polytungstate solution (SPT) (NaPT; Sometu, Belgium) at a density of 1.8 g cm^{-3} and shaken gently for 30 min at 100 rpm on a reciprocated shaker. Tubes were centrifuged at 1874 RCF for 30 min and floating material was removed by vacuum filtration onto 0.45 μm cellulose nitrate filters. To remove residual SPT from the POM_{free} fraction, filters were rinsed with milliQ water. Complete SPT removal was assumed when the conductivity of the rinse water fell below <50 μs cm^{-1} (Measured using a calibrated Jenway 4510 probe). Material was then washed from the filters into individual aluminium foil trays. Liberation of the $\text{POM}_{\text{occluded}}$ fraction was carried out using ultra sonication (Sonics Vibracell CV18 probe). Another 40 ml SPT was added to each tube and vortexed for 1 min to re-suspend the soil pellet. Sonication was then performed for 5 min per sample to achieve a minimum power of 50 W (Poeplau and Don, 2014). Tubes were submerged in an ice bath during sonication to maintain sample temperature at <40 °C (Schrumpf et al., 2013). Tubes were then centrifuged, material filtered and rinsed with milliQ as described above. As the mass of $\text{POM}_{\text{occluded}}$ was generally small, the $\text{POM}_{\text{occluded}}$ fraction was combined with the POM_{free} fraction in an aluminium foil tray to form one complete POM fraction, oven dried at 40 °C and weighed. The centrifuge tubes containing the remaining MAOM fraction were refilled with milliQ water and centrifuged at 1874 RCF for 2 h. This step was repeated 4 times until the conductivity of the supernatant was <50 μs cm^{-1}. The soil pellet was transferred to a pre-weighed aluminium tray, oven dried at 40 °C and weighed. C associated with fine particles (<20 μm) is more stable than C associated with larger particles (>20 μm) (Hassink, 1997) and is typically used to determine carbon saturation ratios (i.e. the capacity for soils to store additional stable C) (Hassink, 1997; Paterson et al., 2021). Therefore the isolated MAOM fraction was further size separated by wet sieving into clay and fine silt (clay + $_{f}$silt) (<20 μm: hereafter referred to as the fine mineral fraction and used in the calculation of C saturation ratios) and coarse silt and sand ($_{c}$silt + sand) (>20 μm: hereafter referred to as the coarse mineral fraction). 40 ml milliQ water was added to the MAOM fraction, sonicated (as described above) to disperse silt-sized aggregates and passed through a stainless steel sieve with a 20 μm aperture with mechanical agitation. All dried fractions were weighed, ground to a fine powder using a Retsch mixer mill (MM400, Retsch, Dusseldorf, Germany) and analysed for C and N using a Costech ECS4010 elemental analyser (Costech Analytical, Valencia CA, USA). For fractionation mass and C recoveries see Supplementary Information: Tables S2–S4.

2.4. Vegetation surveys

Grassland sward composition was assessed by using a standard 1 × 1 m quadrat at 5 of the soil sampling locations within each grassland field. The percentage cover was recorded for each species of vascular plant present within each quadrat.

2.5. Soil carbon saturation of fine mineral fraction

An upper limit of SOC in the fine mineral fraction for each arable and grassland soil was estimated using the slope of the quantile regression ($y = 0.92x$) established for UK soils by Paterson et al. (2021), which relates the mass proportion of the fine mineral fraction (<20 μm %) to fine mineral fraction OC (g C kg^{-1} soil) for UK grassland soils. A soil C saturation ratio was calculated by dividing the current fine fraction organic C by the estimated maximum fine fraction organic C content. Values < 1 were considered under saturated; 1 represents C saturation and values > 1 were deemed oversaturated.

2.6. Data analysis

SOC stocks were calculated with an equivalent soil mass (ESM) approach using a reference dry soil mass of 2000 Mg ha^{-1} following the method of Gifford and Roderick (2003). This approach was used to control for differences in field soil bulk density that may have arisen due to differences in land management. C stocks for POC and MAOC pools were calculated as above using the C concentration of each fraction multiplied by the mass proportion. Differences in total C stocks between farms was tested using ANOVA with mean soil C stocks per field as the dependent variable and farm as the independent variable. Tukey's honestly significant difference *post-hoc* test was used to report pairwise differences between farms. Curve fitting was performed using linear and nonlinear regression (R Stats Package: *lm* and *nls* functions respectively) to describe the relationship between soil C stocks, % SOC and grassland age at each farm. As soil C stocks may reach a steady-state equilibrium over time as C inputs match rates of decomposition, the best fitting model for each relationship was selected from a linear ($Y = X$), logarithmic growth ($Y = log(X)$) and nonlinear asymptotic regression ($Y = a-(a-b)*exp(-cX)$) model, where a is the maximum attainable Y (horizontal asymptote), b is Y at X = 0 and c is proportional to the relative rate of Y increase while X increases. As R^2 is not a valid measure of goodness of fit for non-linear regression, best fit was assessed across both linear and nonlinear models by selecting models with the lowest standard error of the regression (S). Linear and logarithmic growth curves were only fitted if statistical significance was $p < 0.05$. The relationship between key soil properties (pH, bulk density), % *Lolium perenne* cover and grassland age was tested using linear mixed effects models as implemented in the lme4 package (Bates et al., 2015), including field as a random factor and age, depth (for soil properties) and site as fixed factors. Differences in the % change of grassland soil C stocks and % *Lolium perenne* cover was assessed using ANOVA with grassland age class and site as the independent variables and an interaction term. Tukey's honestly significant difference *post-hoc* test was used to report pairwise differences between age classes. Multiple linear regression was used to predict SOC, MAOC and POC stocks across sites using soil, vegetation, grassland management and climatic variables (soil texture, pH, % *Lolium perenne* cover, N fertilization rate, mean annual precipitation and temperature). Variables were checked for correlation by inspecting variance inflation factors and the best model was then selected by forward and backward stepwise model selection using AIC as a criterion. Relative importance (proportion of variance in the Y variable accounted for by each X variable) of predictors was

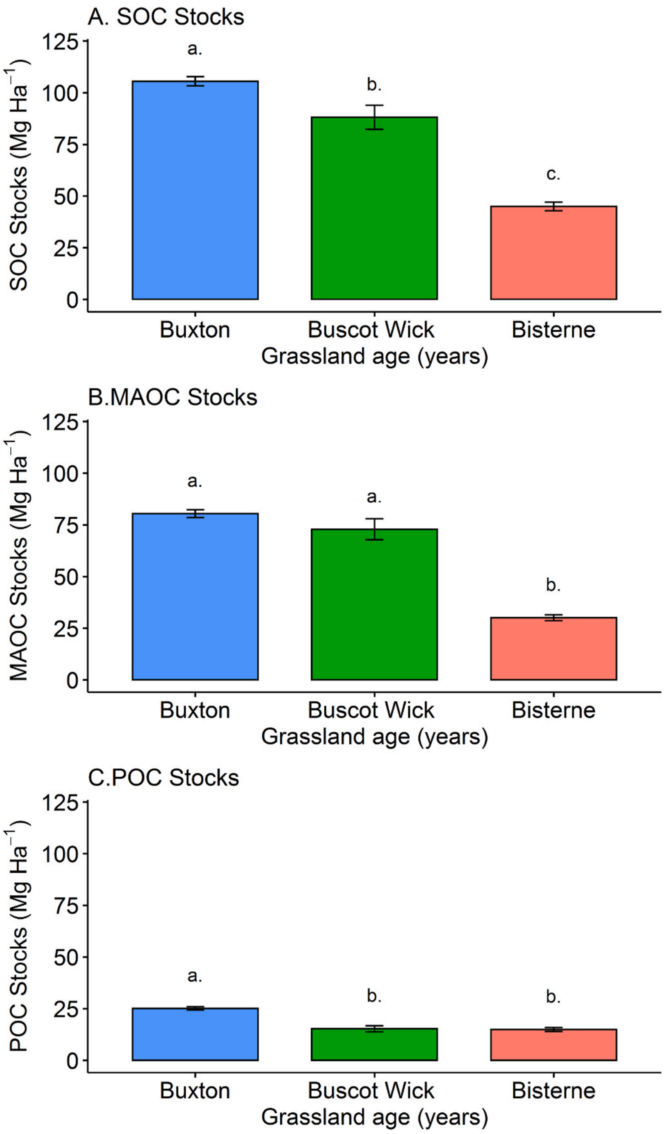

Fig. 2. Grassland SOC (A.), MAOC (B.) and POC (C.) stocks across three surveyed farms in England (Buxton, Buscot Wick and Bisterne). Bars represent means and error bars represent ±1 standard error. Lowercase letters above bars denote significant differences between farms at $p < 0.05$.

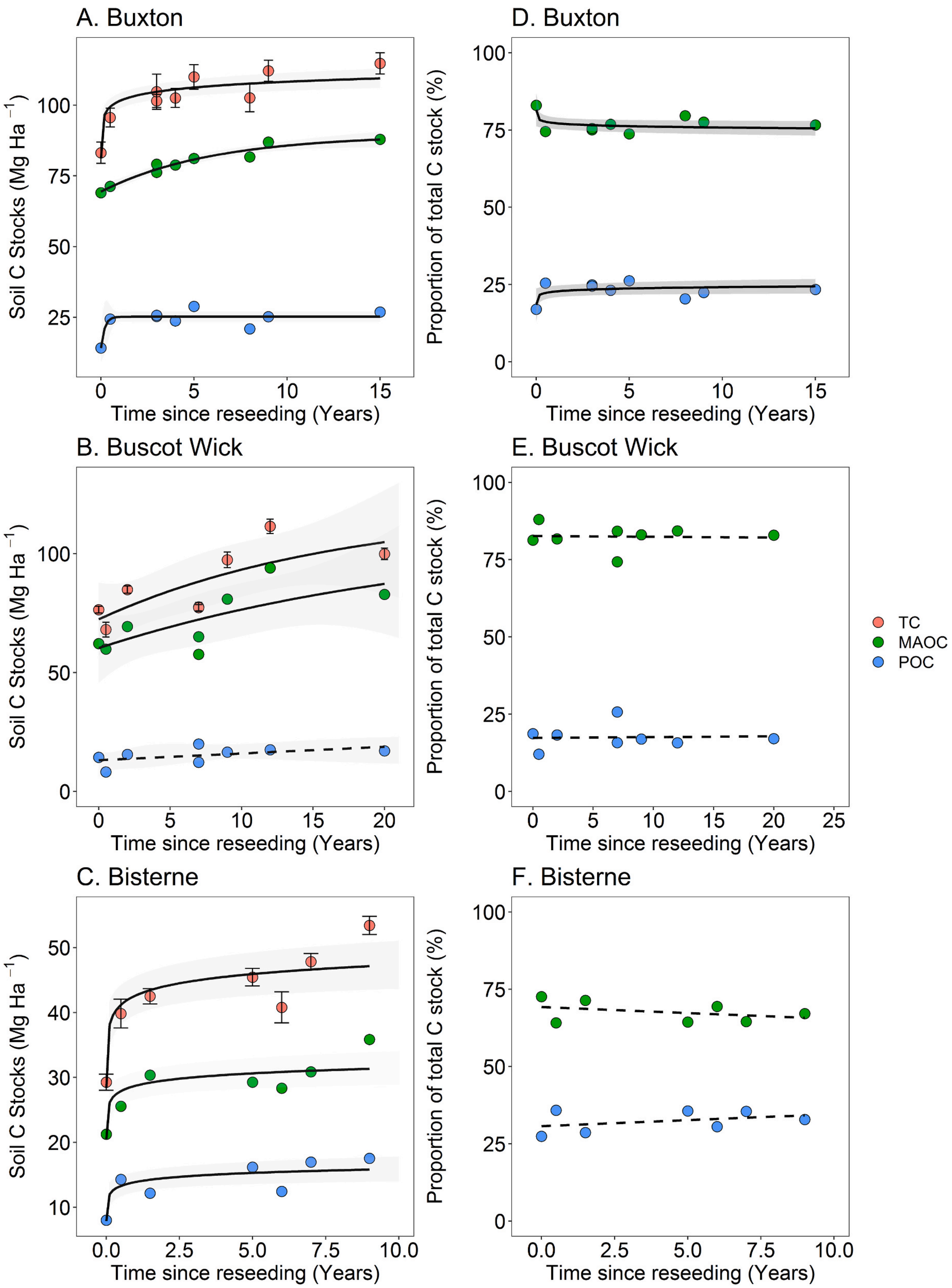

Fig. 3. A–C: Relationship between grassland age and total, particulate and mineral associated C stocks. D–F: The relationship between grassland age and the percentage of total C stocks stored in particulate and mineral associated C pools. Best fit regression lines were fitted to the data from a choice of linear ($Y = X$), logarithmic ($Y = log(X)$) or asymptotic regression ($Y = a-(a-b)*exp(-cX)$) where y = TC, POC or MAOC stocks, x = grassland age, a = the maximum value of y (horizontal asymptote), b = y at x = 0 and c is proportional to the relative rate of Y increase while X increases. Model formulas and *p* values for linear regressions are as follows: Panel A: TC: $y = 2.95\log(x)+101.5$, $p < 0.001$; MAOC: $y = 89.52-(89.52-69.40)*exp(-1.79x)$; POC: $y = 1.20\log(x)+23.3$, $p < 0.001$; Panel B: TC: $y = 119.18-(119.18 -72.41)*exp(-2.83x)$; MAOC: $y = 109.21 -(109.21-60.33)*exp(-3.21x)$; Panel C: TC: $y = 2.06\log(x)+42.6$, $p < 0.01$; MAOC: $y = 1.19\log(x)+28.7$, $p < 0.05$; POC: $y = 0.87\log(x)+13.9$, $p < 0.05$; Panel D: MAOC: $y = -0.62\log(x)+77.3$, $p < 0.05$; POC: $y = 0.62\log(x)+22.7$, $p < 0.05$. Grey shaded areas represent the 95% confidence envelope. Dashed lines indicate non-significant relationships ($p > 0.05$).

calculated by partitioning R^2 and averaging over orderings of regressors using the *Relaimpo* R package (Grömping, 2006). ANOVA's and multiple linear regression were performed using the *aov* and *lm* functions (R Core Team, 2022) and post-hoc tests were performed using the emmeans R package (Lenth, 2021).

3. Results

3.1. Current SOC stocks

Measured total SOC stocks varied between farms (Fig. 2A). Total SOC stocks varied from 29.3 to 114.8 Mg C ha^{-1} with a median of 83.2 Mg C ha^{-1} (Supplementary Information: Table S1). Buxton had higher SOC stocks than Buscot Wick ($p < 0.05$) whilst Bisterne had significantly lower total SOC stocks than either Buxton or Buscot Wick ($p < 0.001$) (Fig. 2A). MAOC stocks also varied between sites (Fig. 2B) ranging from 21.2 to 94.0 Mg C ha^{-1} with a median of 69.0 Mg C ha^{-1} (Supplementary Information: Table S1). Bisterne had significantly lower MAOC stocks than either Buxton or Buscot Wick ($p < 0.001$) but there was no difference between Buxton and Buscot Wick (Fig. 2B). POC stocks were lower than MAOC stocks across all farms and varied from 8.0 to 28.9 Mg C ha^{-1} with a median of 17.1 Mg C ha^{-1} (Supplementary Information: Table S1). Buxton had higher POC stocks than Bisterne or Buscot Wick ($p < 0.001$) but there was no difference between Bisterne and Buscot Wick (Fig. 2C).

3.2. Relationship between SOC stocks, grassland age, soil properties and sward composition

SOC, MAOC and POC stocks were related to the age of the grassland sward although the strength of the association varied between surveyed farms. Across farms, the relationship between soil C stocks and grassland age was best described by logarithmic or asymptotic regression growth curves (Fig. 3A–C). Thus, in either case the greatest increases in SOC stocks were observed soon after arable to grassland conversion followed by progressively slower SOC accumulation. After 1 year following conversion from arable cropping, grassland SOC stocks increased by 18.3 (15.6–21.0 95% CI), 2.69 (−9.5-14.9 95% CI) and 13.3 (10.4–16.3 95% CI) Mg C ha^{-1} year at Buxton, Buscot Wick and Bisterne farms respectively. From 1 to 4 years after reseeding, grasslands SOC stocks increased by an average of 1.36 ± 0.62, 2.39 ± 0.14 and 0.96 ± 0.44 Mg C ha^{-1} year at Buxton, Buscot Wick and Bisterne farms whilst from 5 to 8 years after reseeding, grassland SOC stocks increased more slowly, accumulating SOC at an average rate of 0.51 ± 0.12, 1.95 ± 0.15 and 0.36 ± 0.08 Mg C ha^{-1}. At Bisterne, the relationship between grassland age, SOC, MAOC and POC stocks followed logarithmic growth indicating an initial period of rapid increase in C stocks across soil fractions followed by progressively slowing but unbounded growth (Fig. 3C). At Buxton, SOC and POC stocks also followed logarithmic growth but MAOC stocks followed asymptotic growth. This indicates that the relative rate of growth in MAOC stocks was maximum when age = 0, decreased as age increased and was bound by an upper limit (Fig. 3A). At Buscot Wick SOC and MAOC stocks both followed asymptotic growth but POC stocks were not related to grassland age Fig. 3B). Both soil pH and bulk density increased with depth (0–15 to 15–30 cm) (pH: $p < 0.001$, Bulk density: $p < 0.001$) but were not related to grassland age (pH: $p = 0.98$, Bulk density: $p = 0.89$) (Supplementary Information: Table S1, Figs. S1A–C). % *Lolium perenne* cover was also not related to grassland age ($p = 0.17$) (Supplementary Information: Figs. S1D–F).

The proportion of SOC stocks stabilised as POC and MAOC was not related to grassland age although at Buxton, conversion from arable to grassland appeared to rapidly reduce the proportion of C stored as MAOC and increase the proportion stored as POC (Fig. 3D–F). When expressed as a percentage change relative to co-located arable fields planted with winter wheat, total SOC stocks in older grasslands (9+ years) had a greater percentage increase (43.2 ± 8.3%) relative to young grasslands (21.0 ± 6.0%) (0–4 years) across all surveyed fields ($p < 0.001$) (Fig. 4A). Mean % cover of *Lolium perenne* across surveyed grasslands was 71.1 ± 3.2%. There was no difference in the % cover of *Lolium perenne* between grasslands of different age classes (0–4, 5–8 or 9+ yrs) (Fig. 4B).

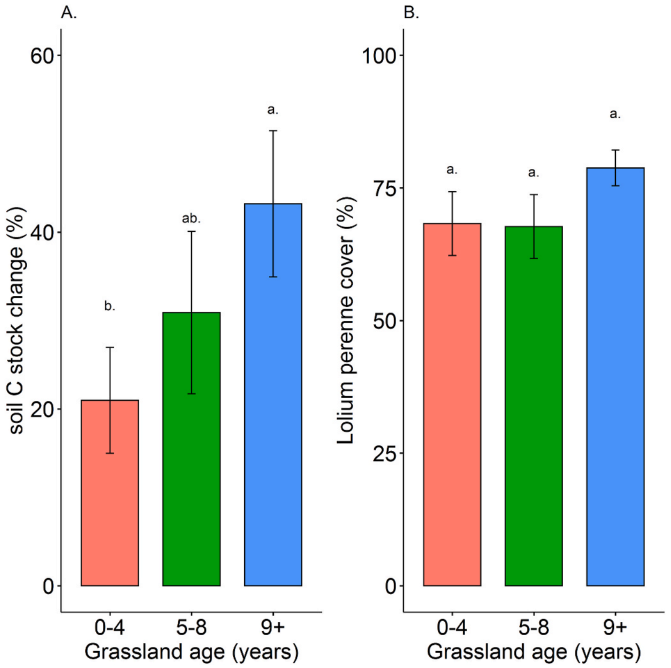

Fig. 4. Percentage change in grassland soil C stocks relative to co-located arable fields planted with winter wheat (A.) and *Lolium perenne* cover in relation to grassland age (B.). n = 7 (0–4 years), n = 7 (5–8 years), n = 6 (9+ years). Lowercase letters denote significant differences between groups.

3.3. SOC concentrations and C saturation deficits

SOC concentrations increased with grassland age at 0–15 cm with the relationship best described by a logarithmic or linear fit (Fig. 5A–C). At 15–30 cm SOC concentrations increased linearly with grassland age at Buxton but no relationship was observed for Buscot Wick or Bisterne (Fig. 5A–C). C saturation deficits at 0–15 cm appeared to decrease with grassland age (Fig. 5D–F) although the strength of the curvilinear relationship varied across farms. Considering the oldest grasslands sampled at each farm, C saturation deficits at 0–15 cm were 38.0 g C kg^{-1} (15 years), 2.7 g C kg^{-1} (20 years) and 4.5 g C kg^{-1} (9 years) at Buxton, Buscot Wick and Bisterne respectively. At 15–30 cm C saturation deficits were larger at 48.6 g C kg^{-1} (15 years), 31.4 g C kg^{-1} (20 years) and 10.9 g C kg^{-1} (9 years) (Fig. 4D–F). C saturation deficits were generally highest in fine textured silty loam soils (Buxton) relative to loam (Buscot Wick) and sandy loam soils (Bisterne) and at 15–30 cm relative to 0–15 cm soil depth (Fig. 6).

3.4. Prediction of soil C stocks

SOC and MAOC stocks were best predicted by the % volume of silt and clay (<53 μm) determined by laser granulometry (S + C), the log of grassland age and mean annual precipitation (MAP) (Table 2). S + C content alone predicted 68% of variation in SOC stock with grassland age and MAP accounting for an additional 11 and 13% respectively. S + C content was also the strongest predictor for MAOC stocks (72%) with grassland age and MAP explaining further variation (grassland age: 8%, MAP: 11%) (Table 2). POC stocks were predicted by a combination of S + C, the % cover of *Lolium perenne* (% Lolium) and MAP. Coefficients and model statistics are presented in Table 2.

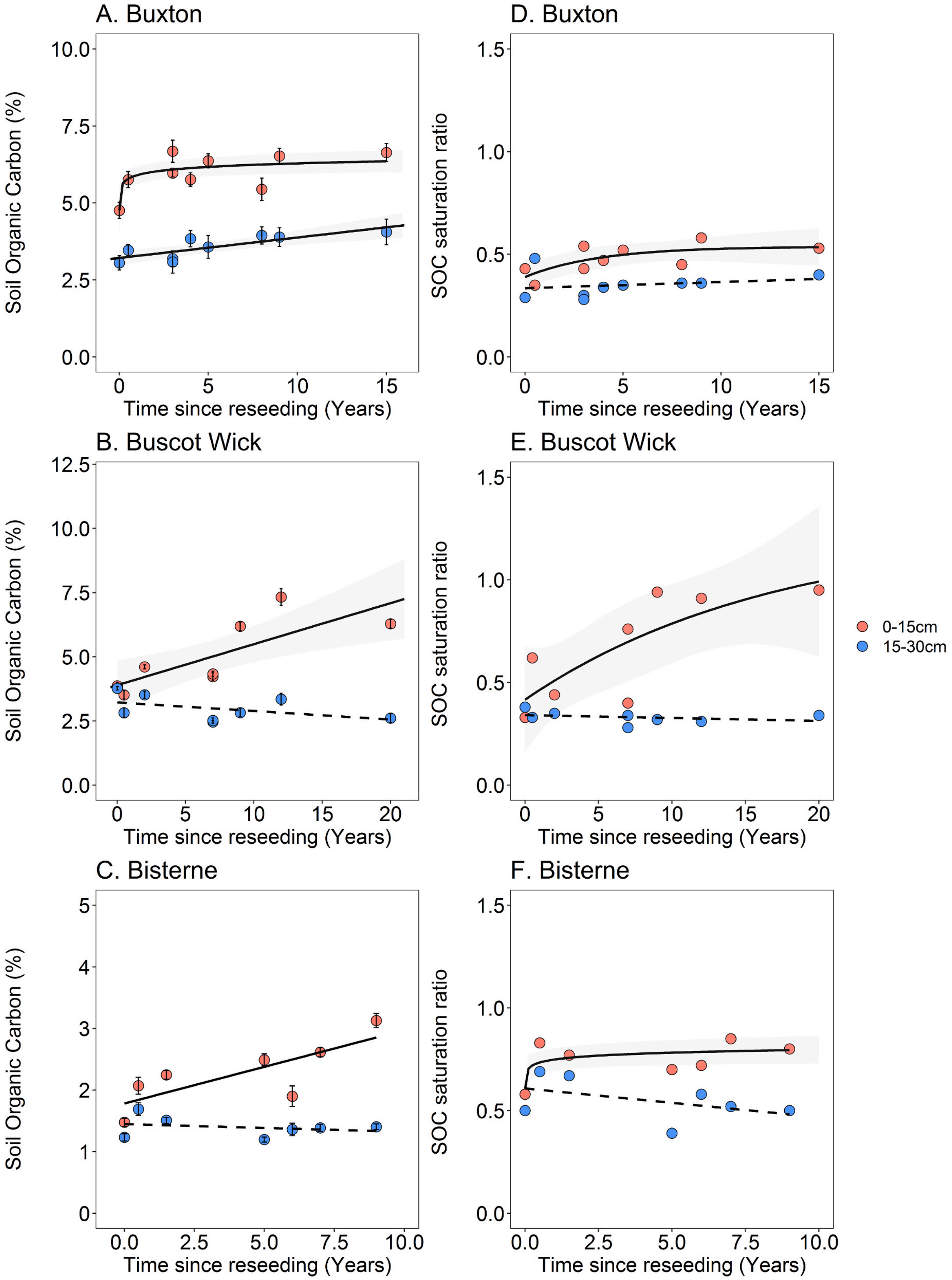

Fig. 5. A–C: Relationship between grassland age and % SOC in topsoil (0–15 cm) and subsoil (15–30 cm). D–F: The relationship between grassland age and the C saturation ratio of the fine mineral fraction OC. Point error bars for panels A–C represent ±1 standard error. Best fit regression lines were fitted to the data from a choice of linear (Y = X), logarithmic ($Y = log(X)$) or asymptotic regression ($Y = a-(a-b)*exp(-cX)$) where y = %SOC or C saturation ratio, x = grassland age, a = the maximum value of y (horizontal asymptote), b = y at x = 0 and c is proportional to the relative rate of Y increase while X increases. Model formulas and p values for linear regressions are as follows: Panel A: 0–15 cm: $y = 0.17\log(x)+5.9$, $p < 0.01$; 15–30 cm: $y = 0.07x+3.2$, $p < 0.01$; Panel B: $y = 0.16x+3.9$, $p < 0.05$; Panel C–$y = 0.12x+1.78$, $p < 0.05$; Panel D: $y = 0.54-(0.54-0.39)*exp(-1.33x)$, Panel E: $y = 1.24-(1.24-0.42)*exp(-2.82x)$, Panel F: $y = 0.02\log(x)+0.75$, $p < 0.05$. Grey shaded areas represent the 95% confidence envelope. Dashed lines indicate non-significant relationships ($p > 0.05$).

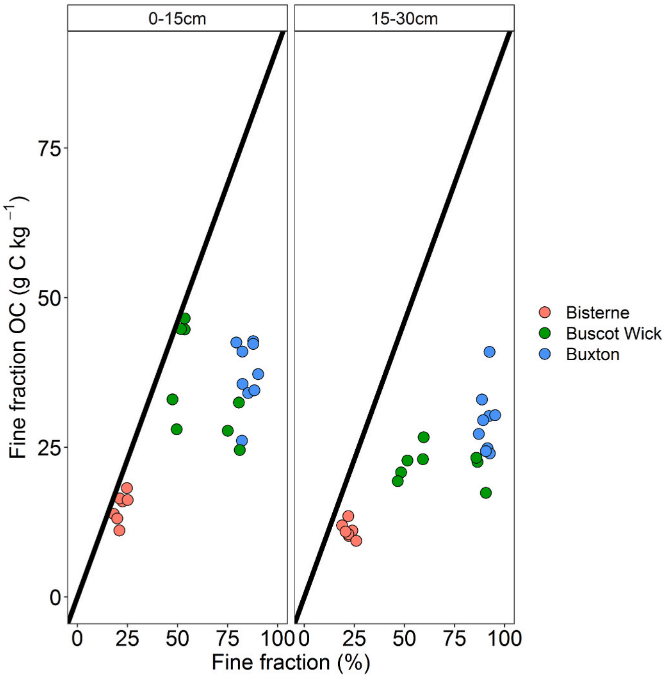

Fig. 6. Measured fine-fraction organic carbon (g C kg^{-1} soil) in relation to the mass proportion of the fine fraction of a soil sample (%). The black line represents the quantile regression analysis (QR) at the 90th percentile established by Paterson et al. (2021) for determining maximum fine mineral fraction organic C in UK grasslands ($y = 0.92x$). Points below the line indicate under saturated soils whilst points above the line indicate oversaturated soils.

4. Discussion

4.1. SOC stocks increased with grassland age

In agreement with hypothesis 1, topsoil (0–30 cm) SOC stocks were higher in grassland relative to arable and increased with time since grassland reseeding (Fig. 3A–C & 4A). The relationship between SOC stocks and time since reseeding followed curvilinear growth with higher initial SOC stock increases associated with arable to grassland conversion followed by slower accumulation (Fig. 3A–C). This agrees with a recent study across intensively managed European grasslands that reported similar C dynamics in the top 10 cm (Iepema et al., 2022). However, the nature of the curvilinear relationship between C stocks and the reseeding interval varied between farms. Logarithmic growth was observed in SOC and POC stocks of coarse (Bisterne) and fine (Buxton) textured soils indicated rapid C accumulation upon conversion from arable to grassland (Fig. 3A and C). MAOC stocks followed asymptotic growth in finer textured soils (Buxton and Buscot Wick) and logarithmic growth in coarse textured soils (Bisterne) (Fig. 3A–C) indicating a fast initial increase in MAOC stocks upon reseeding for coarse textured soils. This suggests that MAOC was more resistant to mineralization during grassland-arable rotation in fine textured soils due to the greater abundance of highly protective micro aggregates in clay rich soils (Schweizer et al., 2019).

The distribution of soil C also differed between farms as the relationship between SOC concentrations and grassland age varied with depth (Fig. 5A–C). At Buxton, SOC accumulated in both 0–15 cm and 15–30 cm but at Buscot Wick and Bisterne, accumulation occurred only in the top 15 cm. The reason for this difference in C distribution is unclear but may be due to variation in climate, soil depth or texture between farms. Buxton had the highest mean annual rainfall, which may promote translocation of dissolved C to depth. Soils at Buxton were also relatively shallow (as some cores reached the parent material within 30 cm), which may limit plant rooting depth and thus limit redistribution of SOC to soil layers below 30 cm (Ojeda et al., 2018). These soils were also fine textured silty loams, which can have poor drainage, potentially reducing leaching of dissolved organic matter.

4.2. SOC did not accumulate preferentially as MAOM

We hypothesized that SOC would accumulate preferentially in MAOM (Hypothesis 2).

POM is vulnerable to microbial turnover and under constant C inputs should reach a steady state where C inputs = microbial mineralization, whilst C associated with minerals may accumulate over a longer period due to physical protection up to a finite carrying capacity (Hassink, 1997; Lavallee et al., 2020). However, in disagreement with hypothesis 2 there was no clear relationship between the proportion of C stored in MAOM, POM and grassland age, suggesting that C accumulates equally across both MAOM and POM C pools (Fig. 3D–F). This may indicate the importance of aggregate dynamics alongside texture in controlling grassland soil C persistence. Reduced macro aggregate turnover appears to promote SOC accumulation via the stabilization of C (both POM and MAOM) into occluded fractions (King et al., 2019). Although we did not perform aggregate fractionation or distinguish between occluded and non-occluded fractions, our findings suggest that soil C accumulation in older grasslands might partially be controlled by physical protection of C within aggregates. Therefore, management that minimises physical disturbance of soils such as no till reseeding or renovation of swards by slot-seeding or over-sowing may hold potential for maintaining higher steady-state SOC level in UK grasslands.

4.3. SOC saturation deficits were greatest in fine textured soils and subsoils

Previous studies suggest that MAOM in the fine mineral fraction

Table 2
Model coefficients for the best-fit multivariate regressions predicting SOC, MAOC and POC stocks from soil properties and environmental variables. Age = Years since grassland reseeding, S + C = % silt and clay, MAP = mean annual precipitation. Relative importance was calculated by partitioning R^2 and averaging over orderings of regressors using the *Relaimpo* R package.

Response Variable	Explanatory Variables	Estimates	Relative Importance (% of R^2)	95% CI	*p* value	Model R^2
SOC stock	Intercept	39.64		[25.99, 53.28]	<0.001	0.92
	S + C	1.00	0.68	[0.82, 1.18]	<0.001	
	log (Age)	3.00	0.11	[1.75, 4.24]	<0.001	
	MAP	−0.02	0.13	[-0.036, −0.001]	0.04	
MAOC Stock	Intercept	36.47		[24.02, 48.92]		0.90
	S + C	0.93	0.72	[0.77, 1.10]	<0.001	
	log (Age)	2.15	0.08	[1.01, 3.29]	<0.001	
	MAP	−0.03	0.11	[-0.045, −0.013]	0.001	
POC Stock	Intercept	−0.49		[-5.25, 4.26]		0.81
	S + C	0.08	0.24	[0.02, 0.14]	0.009	
	% Lolium Cover	0.10	0.29	[0.06, 0.15]	<0.001	
	MAP	0.01	0.28	[0.00, 0.01]	0.01	

exhibits saturation dynamics (Cotrufo et al., 2019; Stewart et al., 2007). Therefore, soils should have theoretical maximum SOC concentrations in MAOM dependent on the mass fraction of fine soil particles and that the C deficit should decrease as C accumulates. In partial agreement with hypothesis 3, SOC saturation deficits decreased with grassland age in topsoils (0–15 cm) but not in subsoils (15–30 cm) (Fig. 5D–F). This may be due to the root distribution of the predominant grass species *Lolium perenne* as most of its roots are typically found in the top 20 cm of soil under well-watered conditions (Bolinder et al., 2002; Crush et al., 2005; Wedderburn et al., 2010). Roots are the primary source of belowground C in grasslands and through microbial transformation may sorb to mineral surfaces or become stabilised within aggregates (Jackson et al., 2017). Increasing diversity in grassland swards to include deeper rooting species such as chicory (*Chicorium intybus* L.), lucerne (*Medicago sativa* L.) and plantain (*Plantago lanceolata* L.) could thus enhance soil C stocks in subsoils that are currently most C deficient (McNally et al., 2015). Diverse swards may also offer co-benefits such as increased resilience to drought, enhanced productivity, feed quality (Woodward et al., 2013) and increased biodiversity (Savage et al., 2021; Woodcock et al., 2013, 2014). An alternative strategy to enhance SOC stocks in subsoils may be to bring C deficient subsoil to the surface by full inversion tillage (FIT), which could then accrue additional C under conventional ryegrass leys (Lawrence-Smith et al., 2021; Madigan et al., 2022; Whitehead et al., 2018). In New Zealand grasslands this approach increased SOC stocks by 14 t C ha^{-1} in four years (Calvelo Pereira et al., 2017). However, the potential for FIT to increase SOC stocks has not been experimentally tested in UK grasslands and research regarding the effect of FIT on trace gas (CH_4 and N_2O) emissions is required.

The size of the C saturation deficit also varied between soil types with fine textured silty loam soils at Buxton having the largest relative C deficit in topsoils whilst loam and sandy loam soils at Buscot Wick and Bisterne were near saturation (Fig. 5D–F, Fig. 6). This is intuitive as fine textured soils have a higher proportion of fine silt and clay particles to which C can sorb. However, the relationship between C saturation deficit and grassland age was curvilinear, suggesting that stable SOC stocks at Buxton appeared to be approaching a steady-state equilibrium and may thus be limited by factors other than the carrying capacity of the fine mineral fraction. For example, if organic C inputs are in equilibrium with the rate of microbial decomposition of SOC, then an increase in the rate of organic C input is required to achieve maximum SOC concentrations in fine textured soils (Castellano et al., 2015). However, soil texture can also control aggregation dynamics and aggregate stability (Totsche et al., 2018). Therefore, much of the reactive surface area of silt and clay particles in fine textured soils may be contained within stable microaggregates. This is a limitation of C saturation estimates based on regression against the mass proportion of fine minerals and therefore, theoretical maximum SOC concentrations might be overestimated relative to those achievable under field conditions.

4.4. *Grassland age was not linked to sward composition or soil compaction*

Extending the age of grassland leys for enhanced SOC storage is a viable climate change mitigation strategy only if productivity is maintained. We found no evidence of a decline in the proportion of *Lolium perenne* with grassland age (Fig. 4B, Supplementary Information: Figs. S1D–F) suggesting that grassland leys may be extended for climate change mitigation without encroachment of weeds impacting forage production. Swards are also often reseeded to rectify soil compaction from farm operations and poaching from livestock. We did not see any relationship between grassland age and soil bulk density (Supplementary Information: Table S1, Figs. S1A–C) further supporting the notion that reseeding may not always be necessary at 5 year cycles. ~300,000 ha of grassland leys are reseeded annually in the UK (Conijn et al., 2002). Our findings show that these grasslands could instead be maintained, enhancing soil C storage and without negative impacts for sward composition and soil compaction. Minimising reseeding of grassland leys could not only enhance SOC storage but also prevent soil erosion, nutrient leaching and soil organic matter loss, which are key drivers of soil degradation in the UK (Gregory et al., 2015; Peake et al., 2022).

5. Conclusions

Our findings suggest that extending the age of grassland leys is a feasible strategy to increase grassland SOC stocks as older grasslands had higher SOC stocks relative to young grasslands. The capacity for further SOC accrual depended on soil texture and depth with fine textured soils and subsoils (15–30 cm) appearing to hold the greatest potential for additional SOC accumulation. The annual increase in SOC stocks diminished with increasing time since reseeding and should be considered temporary, as arable fields in a typical ley-arable-ley rotation had lower soil C stocks than grasslands on the same soil type. Therefore, where possible grasslands should be maintained continuously to maximise SOC stocks. When sward renovation is necessary, low disturbance methods such as slot-seeding and over sowing should be considered over disruptive mould board ploughing and shallow cultivation to minimize the disruption of soil aggregates, which may promote SOC persistence. However, full inversion tillage during reseeding has potential to maximise SOC stocks in under saturated grassland subsoils.

Credit author statement

Dafydd M. O. Elias: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft Preparation; Writing – review & editing. **Kelly E. Mason**: Investigation. **Katherine Howell**: Investigation. **Nadine Mitschunas**: Investigation **Lucy Hulmes**: Investigation **Sarah Hulmes**: Investigation. **Inma Lebron**: Investigation. **Richard F. Pywell**: Conceptualization; Funding acquisition; Writing – review & editing **Niall P. McNamara**: Conceptualization; Funding acquisition; Writing – review & editing; Project administration.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Niall P. McNamara reports financial support was provided by Arla Foods.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.117465.

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