

Soil organic matter storage in temperate lowland arable, grassland and woodland topsoil and subsoil

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RESEARCH PAPER

Soil organic matter storage in temperate lowland arable, grassland and woodland topsoil and subsoil

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Abstract

Soils are the largest terrestrial pool of organic carbon, with up to 50% of soil organic carbon (SOC) stored below 30 cm. Knowledge of the impact of land use on the mechanisms by which SOC is stored in subsoils is critical to developing and delivering strategies to mitigate climate change. We characterized SOC under arable, grassland, and deciduous woodland land uses in lowland England to determine how land use affects the mechanisms by which topsoil and subsoil SOC are protected. Soil organic matter (SOM) physical fractionation and ammonium oxalate extractable Al, Fe and Mn were analysed to elucidate protection mechanisms. Results revealed that the mineral-free particulate organic matter (fPOM) fraction was significantly greater in both the topsoil and subsoil under woodland than under grassland or arable. The mineral-associated organic carbon (MinOC) fraction was proportionally greater in the subsoil compared with topsoil under all land uses, with arable > grassland > woodland. These findings indicate that land use affects the extent to which SOC is protected, with woodlands containing a higher proportion of carbon that has less protection from decomposition. Subsoil SOC is protected from decomposition by organo-mineral interactions with amorphous Al, Fe and Mn, and may be susceptible to future pH shifts as a result of land use change. This study highlights the need to consider the impact of land use change on SOC, given policy and public interest in woodland planting for climate change mitigation.

KEYWORDS

land use, mineral associated organic carbon, organo-mineral interactions, particulate organic matter, soil depth, soil organic matter

1 | INTRODUCTION

Increasing concentrations of atmospheric CO₂ are currently causing global concern because of their propensity to change our climate (Smith et al., 2016). Soils contain approximately 1500 Gt of organic carbon to a depth of

1 m globally and represent the largest terrestrial pool of organic carbon (Batjes, 1996; Jobbágy & Jackson, 2000). Cultivation and management of agricultural soils and the associated liberation of organic carbon as CO₂ have been cited as major factors contributing to this atmospheric CO₂ increase (Lal, 2018). Recent policy initiatives (e.g.

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4 per 1000) have placed a strong focus on the capacity of soils to sequester carbon and mitigate climate change (Minasny et al., 2017). Changes in land use, particularly conversion of agricultural land, both pasture and arable, to woodland have been recommended as a climate change mitigation strategy to capture and store atmospheric carbon on land (Committee on Climate Change, 2018; IPCC, 2019).

Soil science has undergone a revolution in our understanding of the mechanisms by which organic matter becomes stable soil organic matter (SOM) in soils. Soil carbon models (e.g. RothC, CENTURY, DNDC) that are currently used in global biogeochemical simulations base their processes upon the assumption that stable SOM results from the chemical recalcitrance of biopolymers, which have a very high mean residence time in soils (Dungait et al., 2012). However, the evidence for the persistence of recalcitrant biopolymers in soils is weak (Lehmann & Kleber, 2015). A new understanding is now developing (Schmidt et al., 2011) that proposes microbial products are the precursors of stable SOM (Cotrufo et al., 2013) and that the primary means by which SOM is stabilized (Schmidt et al., 2011) is because of physical protection through aggregate formation (Six et al., 2000), chemical protection because of sorption on soil surfaces (Kaiser & Guggenberger, 2000) or a combination of both (Hernandez-Soriano et al., 2018). The use of physical fractionation methods to quantify the proportion of SOM which is protected from decomposition in topsoils because of aggregates or sorption, and the portion that is unprotected, has become popular in the literature (Duddigan et al., 2019; Plaza et al., 2012; Six et al., 2002; Sohi et al., 2001). Attempts have been made to attribute the quantity of carbon in each fraction to the pools defined by a SOM turnover model, each of which have different degrees of stability defined by different rates of decay (Skjemstad et al., 2004; Zimmermann et al., 2007).

While observations of decreasing C/N ratio down soil profiles has previously been interpreted as the presence of SOM that has undergone greater microbial processing with increasing soil depth, there is new evidence to suggest that this is actually because of soil mineralogy changing with depth in soils (Kramer et al., 2017). Although there is a well-established relationship between the particle size of soils and the capacity to store soil organic matter (Angst et al., 2018; Dexter et al., 2008), Kleber et al. (2007) highlight that the distribution of SOM over the surface of soil surfaces is not homogenous and is instead concentrated in patches. It therefore seems that organo-mineral interactions on soil surfaces of some minerals act as 'nuclei' for carbon retention in soils and therefore play a particularly important role in chemically protecting mineral-associated organic carbon from decomposition (Coward et al., 2018; Torn et al., 1997). Short-range-order minerals

appear to play a particularly important role (Kramer et al., 2012), including aluminium (Al)-bearing minerals such as allophane (Hanudin et al., 2002), manganese (Mn)-bearing minerals such as birnessite (Li et al., 2021) and iron (Fe)-bearing minerals such as ferrihydrite (Yu, Xiao, et al., 2017; Yu et al., 2017b). The chemical availability of mineral phases on soil surfaces is commonly quantified using chemical extractions, including the use of acidic ammonium oxalate to dissolve and chelate amorphous and short-range-order minerals (Coward et al., 2017).

Global surveys have typically focused on the 0–30 cm topsoil layer (Bell et al., 2011), but it is now known that as much as 50% of soil carbon is stored below 30 cm (Lal, 2018; Rumpel & Kögel-Knabner, 2011). This is because the volume of subsoil is far greater than the topsoil, even though the concentration of organic carbon in the subsoil is lower than topsoil. Many of the recent insights regarding the stability of organic carbon in soils described above have been inferred because of measurements made on topsoil, and it is not known to what extent these mechanisms underpin the stability of SOM in subsoils. Therefore, the factors responsible for the stability of organic carbon stored in deeper soils is a key knowledge gap. The literature contains conflicting reports with respect to the stability of SOM in subsoils and the extent to which it is microbially processed and physically or chemically protected from decomposition (Rumpel & Kögel-Knabner, 2011). Some papers indicate that subsoil SOM is more stable than in the topsoil because of a strong interaction with clay mineral phases and protection in microaggregates (Torres-Sallan et al., 2017), whereas other papers indicate that deep SOM can be easily primed by fresh organic carbon (Fontaine et al., 2007) supplied by earthworm mucus (Hoang et al., 2017) or plant root exudates (Shahzad et al., 2018). Since soil biota plays an important role in the formation of soil aggregates (Lehmann et al., 2017), it might be assumed that physical protection mechanisms play less of a role in the subsoil, compared with the topsoil. However, occlusion of SOM by aggregates has been shown to play an important role in subsoils (Moni et al., 2010) and addition of fresh plant material to subsoils was found to generate more water stable macroaggregates than addition of the same quantity of plant material to topsoil (Poirier et al., 2014). The reason suggested for this greater subsoil aggregate creation was because of a greater abundance of unsaturated mineral surfaces in the subsoil. Therefore, it is likely that sorption of SOM facilitates aggregation in the subsoil (Yu, Xiao, et al., 2017; Yu, Zhang, et al., 2017) and we expect that sorption, particularly to short-range-order minerals (Chevallier et al., 2019), will play a greater role than physical protection mechanisms in the subsoil.

The conversion of land from natural ecosystems to agricultural lands has resulted in the global loss of 133

Pg of C from the soil, and it is widely acknowledged that these degraded, carbon-depleted lands offer the greatest potential to store significant additional quantities of carbon by implementing restorative land management practices (Lal, 2018; Sanderman et al., 2017). The influence of land use change, because of the alteration of management practices such as ploughing, on the physical fractions of topsoil SOM is well understood (Leifeld & Kögel-Knabner, 2005; Miller et al., 2019). However, the impact that land use and land management change have on subsoils is more likely to be influenced by the depth and composition of root exudates, the leaching of dissolved organic matter, and the burial of organic matter by soil organisms (Lorenz & Lal, 2005). There is relatively little known about how land use change influences subsoil SOM fractions and how these fractions change over time (Gregory et al., 2014). Thus, there is a need to examine the factors affecting soil carbon storage at depth under different land uses to assess their capacity for further soil carbon sequestration and prevent unintended priming and loss of soil carbon.

To address the knowledge gaps associated with understanding how land use affects the mechanisms that underpin SOM dynamics and whether these mechanisms in topsoils also underpin subsoil SOM dynamics, this paper has three objectives: (i) characterize the organic carbon in soils under woodland, grassland and arable vegetation in England, down the first 1m of the soil profile; (ii) determine the mechanisms of SOC protection in both topsoils and subsoils by employing physical fractionation methods and chemical extractions; and (iii) evaluate how SOM stabilization mechanisms alter with depth and whether they

are affected by land use. We hypothesized a greater importance of chemical protection mechanisms and lower importance of physical protection mechanisms in the subsoil, compared with the topsoil, assuming that chemical recalcitrance of biopolymers provides only short term protection from SOM decomposition.

2 | MATERIAL AND METHODS

2.1 | Field sites

Soil samples were collected in August 2017 from three separate locations within the Loddon Catchment, a tributary of River Thames in the UK (Figure 1). At each location, soils were collected from a deciduous woodland, a permanent grassland and a long-term arable site. All woodland sites were classified as 'Broadleaved, mixed and yew woodland', all grassland sites were classified as 'Improved grassland', and all arable areas were classified as 'Arable and horticulture' on the Centre for Ecology and Hydrology 2015 Land Cover Map (Rowland et al., 2017). Sites were also on the same mudstone geology that is typical of flat low-lying areas of the Thames Valley river terraces (Bloomfield et al., 2011). Two of the locations were the University of Reading's research farms: Sonning Farm and Hall Farm, and the third was farm and woodlands owned by The Vyne, National Trust. All soils had similar silty-clay loam surface texture with clay enrichment in subsoils and are affected by groundwater or waterlogging. Under the original Soil Survey of England and Wales classification, Sonning Farm is an argillic brown

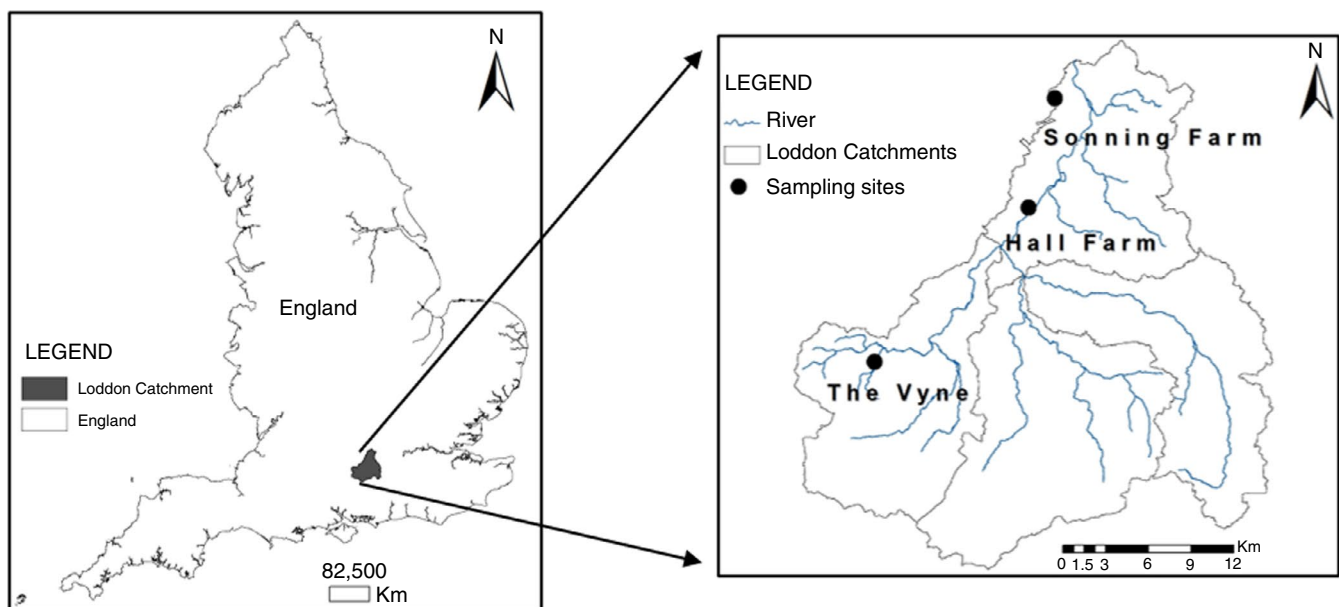


FIGURE 1 Map of soil sampling sites within the Loddon catchment in the UK

earth that is typically freely drained by sometimes affected by groundwater (soil series 0571w Hucklebrook), Hall Farm has argillic gley soils that are permeable soils affected by groundwater (soil series 0841b Hurst), and The Vyne has typical stagnogley soils, which are slowly permeable seasonally waterlogged soils (soil series 0711h Wickham) (Cranfield University, 2021). The Soil Survey of England and Wales classifications have been correlated and reclassified using the World Reference Base (2006, Tier 1 Version) as a Luvisol, a Gleysol and a Planosol, respectively.

2.2 | Sampling design

Five soil samples were collected from every site using a gouge auger, adopting a stratified random sampling strategy. Each core was sampled down to 1 m depth and divided into 10-cm segments. Samples were then bulked into one composite soil sample for each 10 cm depth per site. Therefore, every site yielded 10 composite soil samples (0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm, 50–60 cm, 60–70 cm, 70–80 cm, 80–90 cm and 90–100 cm). With three different land uses (i.e. three sites per location) and three locations (Sonning farm, Hall farm and The Vyne), 90 composite samples were collected overall from a total of nine sites. Soils were air dried and sieved to <2 mm prior to analysis.

2.3 | Laboratory methods

2.3.1 | Soil carbon and nitrogen

Soil organic carbon (SOC) and nitrogen were analysed by dry combustion (Flash 2000 C/N analyzer; Thermo Scientific, Waltham, MA, USA). A subsample was ground to a fine powder using a ball mill and 10 mg weighed into a tin cup before triplicate analysis. 21 replicates of an in-house QC material that is traceable to GBW07412 (certified for nitrogen (N) by State Bureau of Technical Supervision, The People's Republic of China) and AR-4016 (certified for carbon (C) by Alpha Resources Inc. with ISO 17025 accreditation) were run alongside samples with recoveries of $100\% \pm 0.003$ and $98.2\% \pm 0.022$ for N and C, respectively.

2.3.2 | Soil texture

The particle size distribution of soil samples was determined using laser-diffraction (Mastersizer 3000 laser granulometer, Malvern Instruments, Malvern, Worcestershire,

United Kingdom). A subsample (± 5 mg) of 2-mm sieved air-dried soil was put onto clean plastic disc and a few drops of a dispersing agent (3.3% sodium hexametaphosphate + 0.7% sodium carbonate) were added to aid the dispersion of particles, particularly clay minerals. Disaggregation of the sample was achieved using a rubber pestle for up to 1 min before analysis. Because the particle size distribution obtained by laser-diffraction methods differ from those achieved using the classical sieve pipette method, we used the following equations reported by Yang et al. (2015) to convert our particle size distribution data from a volume % basis to a mass % basis:

$$\text{Estimated Clay}_{\text{SPM}} = 2.17 + 5.76 \exp(0.10 \text{ Measured Clay}_{\text{LDM}}),$$

$$\text{Estimated Sand}_{\text{SPM}} = 6.83 + 0.81 \text{ Measured Sand}_{\text{LDM}},$$

$$\text{Estimated Silt}_{\text{SPM}} = 100 - \text{Estimated Clay}_{\text{SPM}} - \text{Estimated Sand}_{\text{SPM}},$$

where Clay_{SPM} , Sand_{SPM} , and Silt_{SPM} are clay, sand and silt content determined with the sieve-pipette method, respectively, and Clay_{LDM} and Sand_{LDM} are clay and sand content determined with the laser-diffraction method, respectively.

2.3.3 | Soil pH

Soil pH was determined using a pH electrode (3310, Jenway Ltd., Essex, Cambridge, UK), calibrated using pH 4 and 7 buffer solutions. 10 g of 2-mm sieved air-dried soil was weighed into a 50-ml centrifuge tube and then shaken with 25 ml ultrapure ($>18.2 \text{ M}\Omega\cdot\text{cm}$) water for 15 min before the measurement was made in the soil suspension.

2.3.4 | Soil organic matter fractionation

Sub-samples of soil at depths representative of topsoil (0–10 and 20–30 cm) and subsoil (50–60 and 90–100 cm) underwent organic matter fractionation using the method developed by Plaza et al. (2012) and Plaza et al. (2013) to obtain the following four fractions; mineral-free particulate OM (fPOM) located outside aggregates (i.e. not protected from decomposition by physical or chemical mechanisms), intra-macro-aggregate organic matter (iMacro), intra-micro-aggregate organic matter (iMicro) and mineral-associated organic carbon (MinOC) that is chemically protected from decomposition by adsorption to mineral surfaces.

Air-dried 2-mm sieved soil was mixed with 80 ml of 1.85 g ml^{-1} density sodium polytungstate (SPT) in a 250-ml centrifuge bottle. The mass of soil extracted was different

for each sample but amounted to 2 mg soil organic carbon per sample (Table S1), based on the prior analysis of C described in section 2.2.1. Therefore, every tube contained the same ratio (2:80, w/v) of SOC to SPT. This adaptation to the published method was made because subsoils contain much lower SOC than topsoils.

The tubes were shaken for 30 s on an end-over-end shaker at 60 rpm and then centrifuged at 2500 g for 30 min. The supernatant containing the fPOM was immediately transferred after centrifugation into a 100-ml polypropylene bottle and filtered using a Buchner vacuum filtration apparatus through a pre-weighed glass fibre filter (GF/A Whatman, UK) to obtain the fPOM. To obtain the iMacro fraction, the heavy fraction containing macroaggregates remaining in the centrifuge tube was broken up by using a micro-aggregate isolator, as described by Six et al. (2002). The heavy fraction in the centrifuge tube was transferred to the top of a 250- μ m sieve by vortex mixing and rinsing with ultrapure water, immersing in deionized water and shaking with 50 silica beads (4 mm diameter) at 150 strokes per minute on a reciprocating shaker under a continuous, steady deionized water flow of c. 0.2 L min⁻¹ to break up stable macroaggregates, following Six et al. (2000) and Six et al. (2002). Micro-aggregates and other soil components <250 μ m flushed through the sieve were transferred to a beaker. Shaking was stopped after c. 5 min, while ensuring that the water below the 250- μ m sieve had run clear and all macro aggregates were broken. The fraction flushed through the 250- μ m sieve and the fraction remaining over the sieve were dried under a heat lamp in a fume cupboard. Both fractions were recombined and gently transferred into a 200-ml polypropylene centrifuge tube together with the filtrate from the first step (SPT solution). The tube was shaken for 30 s on an end-over-end shaker at 60 rpm and centrifuged at 2500 g for 45 min. The supernatant containing the iMacro fraction was obtained by pipetting and filtering, as described above. To obtain iMicro fraction, the heavy fraction was re-suspended in the SPT solution (collected from the previous step) and microaggregates dispersed by sonication with an energy input of 1500 J per gram of soil. Again, the tube was shaken for 30 s on an end-over-end shaker at 60 rpm and centrifuged at 2500 g for 60 min. After centrifugation, floating material, representing the iMicro fraction, was gently pipetted and filtered, as described above. Lastly, the MinOC fraction was obtained by transferring the heavy fraction (containing MinOC) to a pre-weighed petri dish and dried under a heat lamp in a fume cupboard prior to C and N analysis. At each filtration step, all the pre-dried and pre-weighed GF papers were carefully removed from the Buchner filtration apparatus and dried overnight at 70°C and then weighed. The dried fractions and the GF papers were ball milled and analysed for C and N by dry combustion (Flash 2000 C/N analyzer; Thermo Scientific).

2.3.5 | Mineral availability analysis

The availability of amorphous Al, Fe and Mn oxide minerals in subsamples of soil that represent the surface layer (0–10 cm and 20–30 cm) and subsoil layer (50–60 cm and 90–100 cm) of each land use were analysed using the ammonium oxalate extraction method reported by Loeppert and Inskeep (1996). An ammonium oxalate solution was made by dissolving 49.74 g ammonium oxalate ((NH₄)₂C₂O₄·H₂O) and 25.22 g oxalic acid (H₂C₂O₄) in to 1 L of ultrapure (>18.2 M Ω .cm) water. Because the pH of the solution was 3.01, it was not necessary to adjust to pH 3.0. 0.5 g of ball-milled soil samples were weighed into aluminium foil wrapped 50 ml polypropylene centrifuge tubes, followed by the addition of 30 ml of the ammonium oxalate solution. All samples were then placed on an end-over-end shaker for 2 h in the dark. Samples were then centrifuged at 3000 rpm for 15 min, and the supernatant carefully decanted into another centrifuge tube. Supernatants were analysed using Inductively Coupled Plasma-Optical Emission Spectroscopy (PerkinElmer 7300 ICP-OES, PerkinElmer Inc., Waltham, Massachusetts, USA) for Fe, Al and Mn after a 100-times dilution.

2.4 | Statistical analysis

All statistical analyses were performed with Minitab version 18.0. using mixed-effects models (MEMs), applying restricted maximum likelihood (REML) for variance estimation and the Kenward–Roger degrees of freedom approximation. The response variables investigated were total C, total N, C:N ratio, %clay, %sand, %silt, median particle diameter, pH, fPOM, iMacro, imicro, MinOC, and ammonium oxalate extractable Al, Fe, and Mn. Our primary interest was the effect of land use (grassland, arable or woodland) and soil depth on soil properties; thus, soil land use and soil depth were the two main fixed effects. Soils from each land use were collected from each location. As each location is on a similar, but slightly different, soil type, the effect of soil type and location were represented in one single random effect called 'location' because, although they cannot be separated, they are included because they may have an effect on the overall spatial variation between sampling locations. Within each sampling site, soils were bulked to produce a composite sample, as described in detail above, such that only one composite sample value for each land use for each soil depth for each farm was available, with $N = 3$ for each land use across the three locations. Therefore, we were able to compare the effect of land use ($N = 3$) and depth across three different locations and examine whether the location affected any of the variation in soil properties itself. Where a significant ($p < .05$) influence of land use or depth on a

measured parameter was detected by a MEM, a least significant difference (LSD) test ($p < .05$) was performed to compare effects of land use and soil depth on the measured parameter. Pearson correlations were first conducted to examine relationships between oxalate extractable Al, Fe, and Mn, and mineral associated carbon (MinOC) before a MEM was used to investigate the influence of land use, soil depth, and oxalate extractable Al, Fe and Mn on mineral-associated carbon (MinOC) in the subsoil layers (50–60 and 90–100 cm). Farm location was selected as a random effect and soil depth, land use, and ammonium oxalate extractable Al, Fe, and Mn were selected as fixed effects.

3 | RESULTS

3.1 | Differences in soil properties with depth under different land uses

Soil organic carbon decreased with soil depth under all three land uses and at all three locations (Figure 2; Figure S1) and was significantly ($p < .05$) greater in woodland

(11.99 ± 1.90 g/kg) and grassland (11.51 ± 1.73 g/kg) soils than arable (8.35 ± 1.03 g/kg) soils (Table S2). Soil organic carbon was also significantly ($p < .05$) greater at 0–10 cm depth (29.60 ± 3.40 g/kg) than 10–20 cm depth (19.68 ± 1.53 g/kg), which was, in turn, significantly ($p < .05$) greater than at 20–30 cm depth (14.49 ± 0.97 g/kg). Topsoil (0–30 cm) organic carbon was also significantly greater ($p < .05$) than subsoil (30–100 cm) organic carbon content (Table S3). Mixed-effect model analysis revealed that farm location had no significant influence ($p > .05$) on any of the analysed soil properties (Table S6).

Similarly to carbon, organic nitrogen decreased down the soil profile (Figure 2) and grassland soils (1.27 ± 0.16 g/kg) had significantly ($p < .05$) greater nitrogen concentrations than woodland (1.08 ± 0.14 g/kg) or arable (1.01 ± 0.20 g/kg) soils (Table S2). Soil organic nitrogen at 0–10 cm depth (2.64 ± 0.26 g/kg), 10–20 cm depth (1.89 ± 0.16 g/kg), and 20–30 cm depth (1.53 ± 0.12 g/kg) was all significantly ($p < .05$) different from one another (decreasing with depth), and topsoil (0–30 cm) had significantly greater concentrations of organic nitrogen than subsoil (30–100 cm) (Table S3).

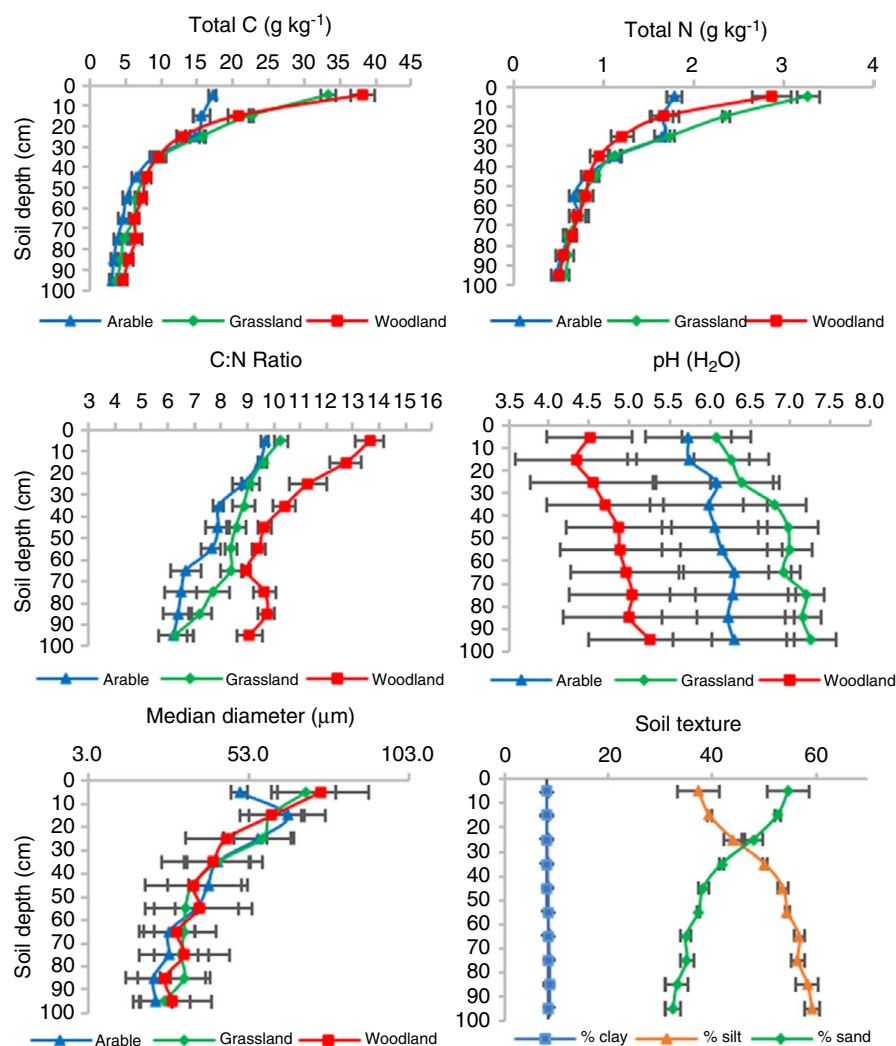


FIGURE 2 Total carbon (g kg⁻¹), total nitrogen (g kg⁻¹), C:N ratio, soil pH (H₂O), median particle size diameter (μm), and soil texture (% clay, silt and sand) at 10 cm depth increments to 1 m under arable, grassland and woodland land uses at Sonning farm, Hall farm, and The Vyne. Points represent mean values from all three sites and error bars represent the standard errors of the mean using the three sites as replicates

The C:N ratio significantly ($p < .05$) decreased down the soil profile (Figure 2; Table S3) and was significantly ($p < .05$) different between different land uses in the order woodland (10.46 ± 0.36) > grassland (8.45 ± 0.29) > arable (7.72 ± 0.31) (Table S2).

Statistical analysis revealed that neither the sand, silt, or clay content, nor the median particle size diameter (Figure 2) differed significantly between different land uses ($p > .05$) (Table S2). There were, however, significant ($p < .05$) differences in soil texture down the soil profile (Table S3; Figure 2; Figure S1). Overall, soil texture of all sampling sites was dominated by sand (50–2000 μm) in the topsoil and silt (2–50 μm) in the subsoil, while the clay fraction was a minor component and was similar in the topsoils and subsoils (Table S3; Figure 2).

Soil pH increased slightly down the soil profile under all land uses, albeit not significantly ($p > .05$) (Figure 2). The average pH, found to be significantly different between land uses ($p < .05$), was in the order, from lowest to highest; woodland (4.81 ± 0.11) < arable (6.09 ± 0.20) < grassland (6.81 ± 0.12) (Table S2).

3.2 | Differences in soil organic matter physical fractionation with depth under different land uses

Physical fractionation of soil organic matter in samples collected from three different land uses, at three different locations, revealed that free particulate organic matter (fPOM), intra-macro-aggregate (iMacro), and intra-micro-aggregate (iMicro) fractions significantly ($p < .05$) decreased both in terms of concentration and relative proportion (except iMacro) down the soil profile (Figure 3). Whereas the concentration of the mineral associated organic carbon (MinOC) fraction significantly ($p < .05$) decreased down the 1 m soil profile, the relative proportion of the carbon that was within the MinOC fraction was significantly ($p < .05$) greater in deeper soil layers compared with other fractions (Figure 3). The two topsoil layers (0–10 and 20–30 cm) had significantly ($p < .05$) greater concentrations of carbon in all SOM fractions than the two subsoil layers (50–60 and 90–100 cm) of the soil profile (Table S4).

Generally, the MinOC accounted for the greatest proportion of the total organic carbon under all three (woodland, grassland, and arable) land uses (Figure 3). Only fPOM concentration was significantly ($p < .05$) greater in woodland soils than grassland and arable soils (Table S4), with other fractions showing no significant ($p > .05$) difference. Considerably, more carbon was associated with the fPOM and iMacro fractions in woodland than arable and grassland soils, but grassland soils contained more carbon associated with the iMicro fraction.

3.3 | Relationship between soil mineral availability and mineral associated soil organic carbon (MinOC) in subsoil

Ammonium oxalate extractable Fe and Mn were slightly lower in the two subsoil layers (50–60 and 90–100 cm) than the two topsoil layers (0–10 and 20–30 cm), while extractable Al was greater in the subsoil. Soil from the Vyne had significantly ($p < .05$) lower concentrations of ammonium oxalate extractable Fe and Mn down the soil profile than soil from Sonning or Hall Farm (Table S5). In subsoils, MinOC increased significantly ($p < .05$) with increasing concentrations of Fe and Mn oxides, but not with Al (Figure 4). Our mixed-effects model indicated that subsoil MinOC concentration was significantly influenced by land use, soil depth (i.e. 50–60 or 90–100 cm), and ammonium oxalate extractable Al, Fe, and Mn concentrations (Figure 4). More than 90% of the variability in the MinOC concentration in the subsoil layers could be explained using the random effect (farm location), and these fixed effects used to generate the model (Figure 4).

4 | DISCUSSION

4.1 | Contrasting soil properties under different land uses

Since we found no significant differences in sand, silt, or clay content, nor median particle diameter, between the three different land uses (Table S2), we therefore infer that comparisons made between arable, grassland and woodland soils can be attributed to their land use, since vegetation cover itself is known as a key soil forming factor in its own right (Jenny, 1994), rather than underlying differences across the sites at each location. This approach is often taken when sampling a pre-existing chronosequence on soils with similar texture (Conant et al., 2004; Cui et al., 2014; Marin-Spiotta et al., 2009). The soil texture was also similar between the three locations (Figure S1, Table S6) reflecting similarity in the underlying geology and topography of the locations, despite being classified as belonging to three different World Reference Base Soil Groups.

The greater concentrations of SOC that we observed in woodland and grassland soils, compared with arable soils (Table S2), are most likely because of higher quantity of litter input into woodland soils because of permanent vegetation (Guo & Gifford, 2002; Li et al., 2016), the livestock manure entering grassland soils (Abdalla et al., 2018), and the tillage of arable soils (Haddaway et al., 2017; Meurer et al., 2018). Woodland soils have a slightly greater concentration of SOC in the top 10 cm, likely because of the presence of a litter layer (Del Galdo et al., 2003), whereas grassland soils have

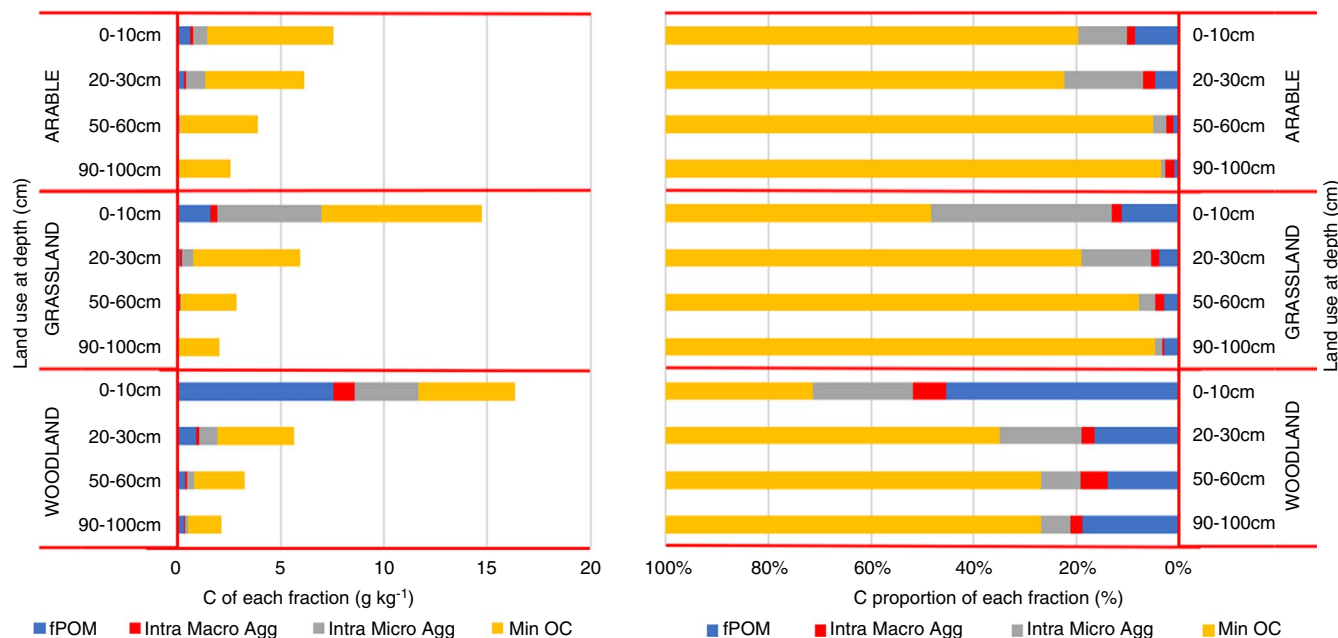


FIGURE 3 Soil organic carbon (C) concentration in g kg^{-1} (left) and relative proportion as a % (right) in each physical fraction of selected soil depths (0–10 cm, 20–30 cm, 50–60 cm, and 90–100 cm) in arable, grassland, and woodland soils from three locations (Sonning Farm, Hall Farm, and The Vyne). Bars represent mean values from all three sites

a slightly greater concentration at 10–30 cm, probably because grasslands have a high root biomass which increases organic matter input to soils, especially in the topsoil (Jobbágy & Jackson, 2000). These data can be used to improve estimates of UK (Bell et al., 2011) and global (Batjes, 1996; Jobbágy & Jackson, 2000) assessments of the impact of land use change on soil carbon stocks.

The greater nitrogen content we measured in grassland soils compared with woodland or arable soils (Table S2) was probably because of the input from the faeces and urine of grazing livestock (Povirk et al., 2001), but could also be explained by higher root biomass in grasslands compared with forest soils (Jackson et al., 1996). Grazing activity increases aboveground and belowground biomass by stimulating more photo-synthetically fixed carbon inputs to belowground roots, leading to increased root exudates and root biomass, which eventually further stimulates nitrogen inputs into soils (Mcsherry & Ritchie, 2013; Zhou et al., 2017). Some studies also show that root litter is more persistent than leaf litter and serves as a mechanism for nitrogen retention in soil (Fujii & Takeda, 2010; Hobbie, 2015). Global analysis by Jackson et al. (1996) indicates that root biomass in grasslands (83%) is higher than temperate deciduous forest (65%).

Our finding that the C/N ratio of soil organic matter was highest in woodland soils, followed by grassland, and then arable soils, is likely because of the impact of vegetation on C/N ratio (Hobley & Wilson, 2016; Jobbágy & Jackson, 2000; Li et al., 2013), since plant litter is the primary source of SOC formation (Li et al., 2016; Lorenz

& Lal, 2005) and the distribution of SOC with depth has a strong relationship with the root depth of vegetation (Schrumpf et al., 2013). Furthermore, the lowest nitrogen concentrations were observed in arable soils, mainly because of leaching of inorganic nitrogen from the profile and repeated growth and harvesting of crops which results in the depletion of soil organic matter (and subsequently soil organic nitrogen) in the absence of manures or nitrogen rich crop residues (Pandey et al., 2018).

Soil pH data indicated that soils under woodland were more acidic than arable or grassland soils (Table S2), which could be attributed to more organic acids (humus layer) in woodland soils as a result of organic matter decomposition (Falkengren-Grerup, 1987; John et al., 2005). There was no significant difference in soil pH with depth, although soil pH tended to increase (not significantly) down the soil profile. The arable and grassland soil pH displayed a similar vertical pH distribution to the sigmoidal model defined by Zhang et al. (2017). They explained lower pH in the topsoil may be because of greater SOM, releasing organic acids upon decomposition, or the uptake of bases by plants in the rooting zone and that higher pH in the subsoil may be because of less SOM, lower disturbance, and fluctuating groundwater.

4.2 | Soil organic matter down the soil profile

Our observations that soil organic carbon and nitrogen concentrations decreased down the soil profile under all

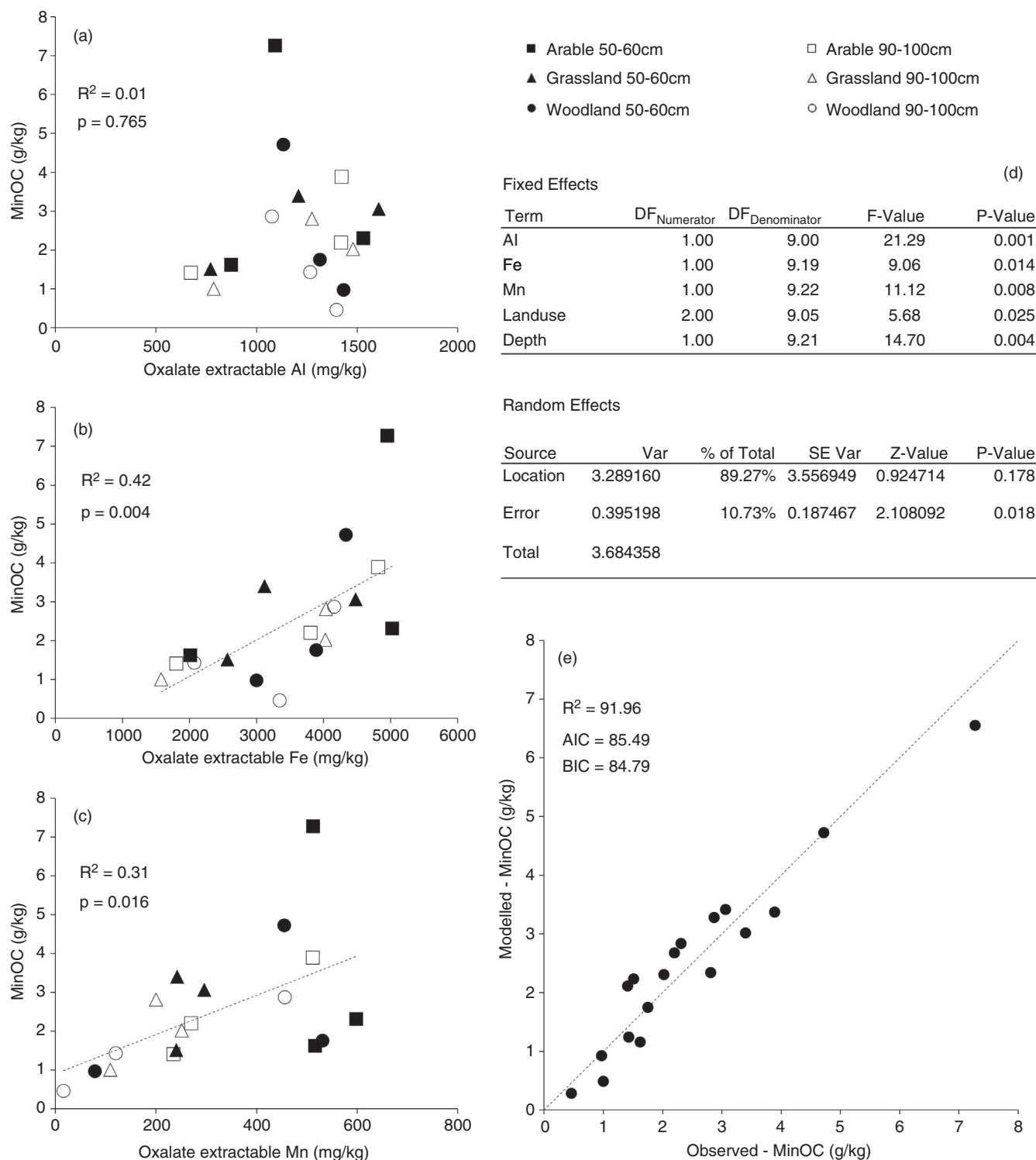


FIGURE 4 Pearson correlations between subsoil mineral-associated organic carbon (MinOC) concentrations and ammonium oxalate extractable aluminium (a), iron (b), and manganese (c), alongside the results of a random mixed effects model (d) that attributes variance in subsoil MinOC concentration to random (farm location) and fixed (oxalate extractable aluminium, iron and manganese, land use and soil depth) effects, accompanied by a plot of the observed and modelled concentrations. $N = 18$. AIC, Akaike's Information Criterion; BIC, Bayesian Information Criterion

land uses support the knowledge that the majority of carbon inputs to soils occurs in the surface horizons (Dorji et al., 2014; Li et al., 2016; Tautges et al., 2019). Whereas topsoil C concentrations were influenced by land use,

subsoil concentrations were similar in woodland, grassland and arable soils, implying that land use change does not considerably alter the concentration of C in the subsoil. This observation challenges the notion that land use

change can result in the loss or additional storage of carbon in subsoils (Lorenz & Lal, 2005; Poeplau et al., 2011) and reflects the uncertainty that stems from a lack of data on subsoil organic matter dynamics.

C/N ratio is a good indicator of the degree of decomposition and quality of the organic matter held in the soil (Batjes, 1996). Our observation of decreasing C/N ratio down the soil profile is attributed to increased accumulation of more extensively decomposed (or microbially altered) organic compounds in the subsoil (Kramer et al., 2017), which is supported by the finding that a greater proportion of the subsoil SOC is chemically protected by association with minerals and there is less particulate organic matter (Figure 3). Organic material with a C/N ratio of approximately 24 is considered optimal for microbial activity, with higher C/N ratios requiring microorganisms to acquire additional N for decomposition to occur (USDA, 2011). Our results indicate that C/N ratios are below 24 in both top and subsoils of all land uses, indicating that the C/N stoichiometry is suitable for organic matter decomposition by microorganisms (Batjes, 1996; Rumpel & Kögel-Knabner, 2011).

4.3 | The physical and chemical protection of soil organic carbon (SOC) down the soil profile

This study revealed clear differences between topsoil and subsoil in relation to the concentration of labile (fPOM), physically protected (iMacro and iMicro), and chemically protected (MinOC) SOC fractions. Most of the mineral-free particulate organic matter (fPOM), which is composed of fresh material including plant litter and root inputs (Dignac et al., 2017; Lal, 2017), is found in the topsoil (Figure 3). This explanation is supported by the finding that deciduous woodland soils, which provide the highest litter inputs, had the greatest fPOM concentration, followed by grassland and arable soils, for which litter inputs are lower (Table S4). However, overall, our results agree with the findings of others (John et al., 2005; Plaza et al., 2012, 2013) that report MinOC as the major SOC fraction in topsoils. We further confirm that this is also the case in the subsoil and that the proportion of SOC that is MinOC tends to increase down the soil profile (Figure 3). This MinOC may have been transported from the topsoil to the subsoil horizon as dissolved organic matter (DOM) with percolating water or exuded at depth by deep pant roots prior to adsorption by soil minerals (Leinemann et al., 2018; Lorenz & Lal, 2005). While >95% of the SOC in the 90–100 cm layer of arable and grassland soils is MinOC, a much lower proportion (<75%) of the SOC in the 90–100 cm layer of woodland soils is MinOC (Figure

3) which indicates that, while woodland subsoil SOC is more accessible for microbial respiration, it may be composed of compounds that are more chemically recalcitrant (Filley et al., 2008) or the trees provide a constant supply of fPOM in the rhizosphere through rhizodeposits (Angst et al., 2016).

Our results imply that the majority of SOC in the subsoil is protected from decomposition because it is chemically associated with mineral surfaces. Fontaine et al. (2007) suggested that SOC in deep soils is protected from decomposition because of a lack of fresh carbon inputs and that changes in land use can result in priming the decomposition of older SOC. Our findings highlight that the role played by minerals, which act as binding agents for forming organo-mineral complexes, becomes more important down the soil profile (Schrumpp et al., 2013; Torres-Sallan et al., 2017), mainly controlled by geochemical interactions (Cagnarini et al., 2019). Soil metal hydroxides act as binding agents for SOC thus protect SOC from decomposer agents (Angst et al., 2018; Yu, Xiao, et al., 2017; Yu, Zhang, et al., 2017). Indeed, we did observe significant positive correlations between the availability of non-crystalline (amorphous) Fe and Mn in the subsoil layers and the concentration of MinOC (Figure 4), in agreement with Rasmussen et al. (2018), and more than 90% of the variability in the subsoil MinOC concentrations can be explained by farm location, land use, soil depth and amorphous Al, Fe, and Mn concentrations (Figure 4).

4.4 | The importance of organo-mineral interactions for subsoil carbon storage

Our observations highlight the importance of understanding organo-mineral interactions in identifying soils with the greatest potential for storing stable subsoil SOC. It has previously been observed that iron-bearing mineral phases, particularly short-range-order mineral phases are strong drivers of SOC sorption on soils (Coward et al., 2018). The chemical mechanisms proposed for this association is the creation of inner-sphere complexes between the hydrophilic functional groups of amphiphiles (molecules with both polar and non-polar regions) and the mineral surfaces of metal hydroxides (Kleber et al., 2007). The SOC associated with these iron-bearing minerals may not be susceptible to microbial decomposition while adsorbed to the mineral surface, but desorption may occur if a reduction in pH occurs below the point of zero net charge of the hydrophilic functional groups of the amphiphile (Bailey et al., 2019). An example provided by Bailey et al. (2019) is pH 4 as the point of zero charge of carboxyl groups, below which the carboxyl group becomes protonated, positively charged and no longer adsorbs to the mineral surface. We

found woodland soils to have lower pH than grassland or arable soils, but this remained, on average, above pH 4 (Figure 2). Overall, soil pH is increasing in the topsoils of all UK habitats, especially arable soils, most likely because of decreasing sulphur emission (Carey et al., 2008). However, further reductions in pH because of land use changes, perhaps because of reversion to acid grassland or heath (Duddigan et al., 2020; Tibbett et al., 2019), or conversion to coniferous plantations (Reich et al., 2005), may result in pH reductions that cause the desorption.

In this study, we demonstrate that, while land use has a minimal impact on the total soil carbon concentration in the subsoil, it does alter the extent to which subsoil organic matter is physically and chemically protected from decomposition. Lavalley et al. (2019) call for a distinction to be made between particulate and mineral associated organic carbon because of their differences in formation, functioning, and persistence. Similarly, Hoffland et al. (2020) plea for SOM fractions to be better linked to soil functions. Particulate organic carbon has a mean residence time of 'years' and is primarily responsible for delivering the soil functions that are characteristic of healthy soils, whereas mineral-associated organic carbon has a mean residence time of 'decades' and represents the long term store of carbon in soils. Likewise, Tautges et al. (2019) call for greater attention to be paid on subsoil carbon when assessing the impact of land use change on soil carbon stocks, since changes in topsoils may not be reflected in subsoils. We highlight here the need to protect mineral-associated soil organic carbon in subsoil layers.

5 | CONCLUSION

It is clearly evidenced that total carbon, total nitrogen, and C/N ratio decrease down the soil profile under woodland, grassland, and arable land uses. Total carbon and C/N ratio were significantly different both between land uses (woodland > grassland > arable) and between topsoil and subsoil. In terms of deep SOC stabilization, SOM fractionation found that free particulate organic matter (fPOM) was mostly found in the topsoil, whereas mineral associated organic carbon (MinOC) fraction dominated the subsoil under all land uses. Although woodlands contained more fPOM in subsoils than other land uses, SOM protection in subsoils is primarily regulated by soil organo-mineral interactions, particularly amorphous Fe and Mn, and may therefore be susceptible to future changes in soil pH that could occur if land use changes, such as conifer plantation, reversion to acid grassland, or heathland restoration, result in subsoil acidification. Our results are novel because they highlight the importance of mineralogy in the protection of SOM in subsoils (within which SOM

dynamics are poorly understood). Future work should focus on identifying soils that have the greatest capacity for storing additional carbon in subsoils and which are at greatest risk of subsoil carbon losses, based on their mineralogy. These advances will inform strategies for developing site-specific land management practices to optimize carbon storage in subsoil horizons.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Mendeley Data at <https://doi.org/10.17632/v8sztp867f.1>

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