Soil Use and Management

WILEY | BRITISH SOCIETY OF SOIL SCIENCE

RESEARCH ARTICLE OPEN ACCESS

Long-Term Effects of Woodland Creation on Soil Carbon Stocks and Aggregate Distribution in Central Scotland

Olivia Azevedo[1] | Elena Vanguelova[2] | Frank Ashwood[3] | Elisa Fuentes-Montemayor[4] | Clare Wilson[4] | Sietse van der Linde[5] | Kirsty J. Park[4]

[1]Forest Research, Northern Research Station, Roslin, Midlothian, Scotland, UK | [2]Forest Research, Farnham, Surrey, England, UK | [3]School of Forestry, University of Canterbury, Christchurch, New Zealand | [4]Biological and Environmental Sciences, University of Stirling, Stirling, UK | [5]Netherlands Food and Consumer Product Safety Authority, Netherlands Institute for Vectors, Invasive Plants, and Plant Health, Wageningen, the Netherlands

Correspondence: Olivia Azevedo (olivia.azevedo@forestresearch.gov.uk)

Received: 5 May 2025 | **Revised:** 9 December 2025 | **Accepted:** 17 December 2025

Keywords: afforestation | chronosequence | forest succession | land use change | mean weight diameter | soil aggregation | soil organic carbon

ABSTRACT

The importance of increasing woodland cover in mitigating climate change is widely recognised, yet quantification of the long-term impact of maturing woodlands on soil structure and carbon stocks remains limited. This study evaluates soil structure and carbon content across a chronosequence of UK woodlands established over the last 160 years and old-growth woodland (250+ years old) compared to pasture sites. Soil organic carbon (SOC) stocks were significantly higher in woodland soils than in pastures, with mature and ancient woodlands containing up to 88%–111% more SOC — primarily due to the development of an organic layer that contributed up to 42% of total carbon stocks. In contrast, differences in carbon within mineral soil layers (0–15 cm, 15–30 cm) were minimal, indicating that afforestation-driven carbon gains are largely restricted to surface horizons. Woodland age had a large effect on carbon concentrations within water-stable aggregates ($\eta^2 = 0.58$, $p < 0.001$), with macroaggregates (> 2000 μm) exhibiting the greatest increases (Cohen's $d = 1.28$). Mid-aged woodlands (31–80 years) displayed particularly even carbon distribution across aggregate size classes, while older stands showed accumulation in finer fractions (< 250 μm), suggesting progressive carbon stabilisation as forests mature. Aggregate stability, measured as mean weight diameter (MWD) from wet-sieving analysis, was significantly higher in woodland surface soils (0–15 cm) compared to pasture ($p < 0.001$), with older secondary woodlands (81–160 years) showing the greatest improvements. Critically, MWD was not a statistically significant predictor of SOC stocks or carbon concentration in mineral soil layers (0–15 cm and 15–30 cm; $p > 0.05$, $\eta^2 < 0.01$), confirming it as an unreliable indicator of carbon status in these systems. This study highlights the essential role of organic layer development in woodland carbon sequestration and provides detailed evidence that woodland age influences not only soil carbon quantity but also its distribution and physical protection within the soil matrix. These findings have practical implications for land management and carbon accounting practices in rewilded and afforested landscapes.

1 | Introduction

Deforestation and forest degradation are pressing environmental issues that contribute significantly to climate change, accounting for approximately 12% of global anthropogenic CO_2 emissions (van der Werf et al. 2009). While the net loss of forests worldwide has decreased since 1990 (due to reduced deforestation, increased afforestation, and natural forest expansion), 4.7 million hectares of forest were still lost annually between 2010 and 2020 (FAO 2020). In Europe, where forest cover has been radically reduced, only 4% is now considered undisturbed primary forest (Maria Sabatini et al. 2018). The net loss of forest area in Europe during the 19th century was estimated to be approximately 60 million hectares, with some countries losing as much as 50%–60% of their forest cover (FAO 2010).

In the United Kingdom, forest cover represents 13% of the total land area, slowly increasing from a record low of 5% at the start of the 20th century (Watts 2006). Forest creation is an essential strategy of the UK government to meet its 2050 Net Zero greenhouse gas emissions target (UK Parliament 2021) by expanding woodland cover in England to 17.5% by 2060 (DEFRA 2022). In Scotland, the government has set an even more ambitious target of increasing woodland cover to 21% of the country's land area by 2032 (Climate Change Committee 2017). However, the success of forest establishment as a climate strategy depends not just on aboveground carbon uptake, but on long-term SOC storage and stabilisation in soils (Jackson et al. 2017).

Soil organic carbon represents the largest terrestrial carbon pool, storing over 1500 Pg globally—more than triple the carbon found in vegetation (FAO 2015). Forest soils contain a dominant share of ecosystem carbon (Nave et al. 2019), with up to 75% of this stored in soil to 1 m depth in UK woodlands (Vanguelova et al. 2013). Soil carbon stability over time is essential for its role in climate mitigation, yet long-term changes in SOC under broadleaved forests remain poorly quantified.

One key determinant of SOC stability is soil structure, especially aggregation. Soil aggregates protect organic matter from microbial decomposition and erosion (Six, Paustian, et al. 2000; Bronick and Lal 2005). According to the aggregate hierarchy model, macroaggregates (> 250 μm) are formed from microaggregates (≤ 250 μm), and increasing macroaggregate stability is associated with better soil aeration, root penetration, and biological activity (Tisdall and Oades 1982; Jastrow et al. 1998).

Aggregate stability is commonly measured by water-stable aggregation (WSA) and expressed as mean weight diameter (MWD)—a widely used soil health indicator (van Bavel 1950; Six, Paustian, et al. 2000; Zhou et al. 2020). Higher MWD values are generally associated with more stable aggregate structures and have been used to track responses to land-use change, including forest establishment (Zhou et al. 2020; Abbas et al. 2021). However, their reliability for predicting SOC content—particularly in temperate broadleaved woodlands—is rarely tested and may vary with woodland age.

Recent studies in tropical and arid forest systems have examined changes in soil aggregation following afforestation (Bougma et al. 2022; de Sinquini Souza et al. 2024), with emerging research highlighting the importance of carbon stabilisation pathways within aggregate fractions during forest succession (Ortiz-Oñate et al. 2022; Xiao et al. 2023). However, few studies have assessed long-term aggregation dynamics in UK broadleaved woodlands. Most UK-based research on land-use change focuses on early-stage planting or coniferous systems, with limited emphasis on *carbon partitioning among aggregate fractions*. The long-term (< 100 years) response of soil aggregation and aggregate-associated carbon to forest succession on former agricultural land remains underexplored.

This study targets that knowledge gap by evaluating changes in soil carbon stocks and structure across a chronosequence of temperate broadleaved woodlands in the Scottish Lowlands, including ancient woodland (> 250 years) and sites aged 20–160 years. These forests were planted on former pasture land and have been used in prior assessments of aboveground biodiversity (e.g., Fuentes-Montemayor et al. 2020), but to date, belowground processes remain unexamined. This study aims to address this significant knowledge gap by providing novel insights into the belowground processes associated with forest creation, particularly examining the long-term changes in soil aggregation and the links between aggregate stability and SOC storage in secondary temperate forests on previously farmed land. Specifically, we address the following research questions, within three broad themes:

1. Woodland creation and soil carbon stocks
   i. How does forest creation impact soil carbon stocks on former agricultural land?
   ii. To what extent does this impact vary with the age of the woodland?
   Anticipated outcome (i): SOC stocks will increase with age, especially during early successional stages (Guo and Gifford 2002; Paul et al. 2002; Laganière et al. 2010).
2. Woodland age and aggregate carbon dynamics
   i. How does woodland age influence aggregate formation and carbon distribution?
   Anticipated outcome (ii): Older woodlands will exhibit enhanced aggregate stability and greater carbon concentration within aggregates (Six, Paustian, et al. 2000; Six et al. 2004; Chenu et al. 2019).
3. Reliability of MWD as an indicator of soil carbon content
   i. Does MWD effectively reflect SOC stocks and stability, and does this vary with woodland age?
   Anticipated outcome (iii): MWD will positively covary with soil carbon content, but the relationship strength may depend on woodland maturity (Six, Paustian, et al. 2000; Bronick and Lal 2005; Lehmann and Kleber 2015; Chenu et al. 2019).

2 | Materials and Methods

2.1 | Terminology Clarification

Throughout this study, the terms 'forest' and 'woodland' are used interchangeably, consistent with UK conventions. In the UK context, 'woodland' typically refers to smaller-scale or semi-natural tree-dominated habitats, but for the purposes of this research (and in much national policy and literature), both terms describe areas of significant tree cover that function as forested ecosystems. The terminology is aligned with the FAO definition, where land with tree canopy cover exceeding 10% qualifies as forest. 'Woodland creation' is a term widely used in the United Kingdom, referring broadly to the establishment of new areas of trees. This includes both *afforestation* (planting trees or creating a forest on land that has not previously supported woodland) and *reforestation* (establishing tree cover on land where woodland was previously present but has since been cleared).

2.2 | Site Selection and Study Design

This study was conducted from 2019 to 2021 as part of the Woodland Creation and Ecological Networks project (WrEN, www.wren-project.com; Watts et al. 2016). Thirty planted broadleaved woodland patches (20–160 years old), 10 ancient woodlands (> 250 years), and five adjacent pasture areas (as controls) were selected across central Scotland (Figure 1). Study sites were located within the Central Lowlands (Midland Valley) of Scotland (approximately 55.8° N to 56.2° N, 3.2° W to 4.2° W), ranging in elevation from approximately 120 to 380 m above sea level (mean ± SD: 250 ± 85 m). The region is characterised by gently rolling terrain typical of the glacially modified rift valley between the Highland Boundary Fault and Southern Uplands Fault. Sites ranged in size from 0.5 to 4.7 ha and were restricted to brown earths (Cambisols) and gleyed brown earths to control for soil type variation. Dominant tree species included *Betula pubescens*, *Betula pendula*, *Acer pseudoplatanus*, *Fraxinus excelsior*, and *Quercus robur*, with species mixtures varying naturally by site. Aboveground stand structure was assessed by quantifying basal area ($m^2 ha^{-1}$), tree density (stems ha^{-1}), and tree diversity (Shannon's *H*) for each woodland age class (see Table S1). Across the chronosequence, basal area increased with woodland age (mean [SD]: ≤ 30 years, 221 [133]; 31–80 years, 151 [87]; 81–160 years, 227 [184]; 250+ years, 248 [160] $m^2 ha^{-1}$), while tree density showed a tendency to decrease in older stands (mean [SD]: ≤ 30 years, 766 [144]; 31–80 years, 455 [262]; 81–160 years, 385 [188]; 250+ years, 448 [278] stems ha^{-1}). Tree diversity varied moderately across age groups.

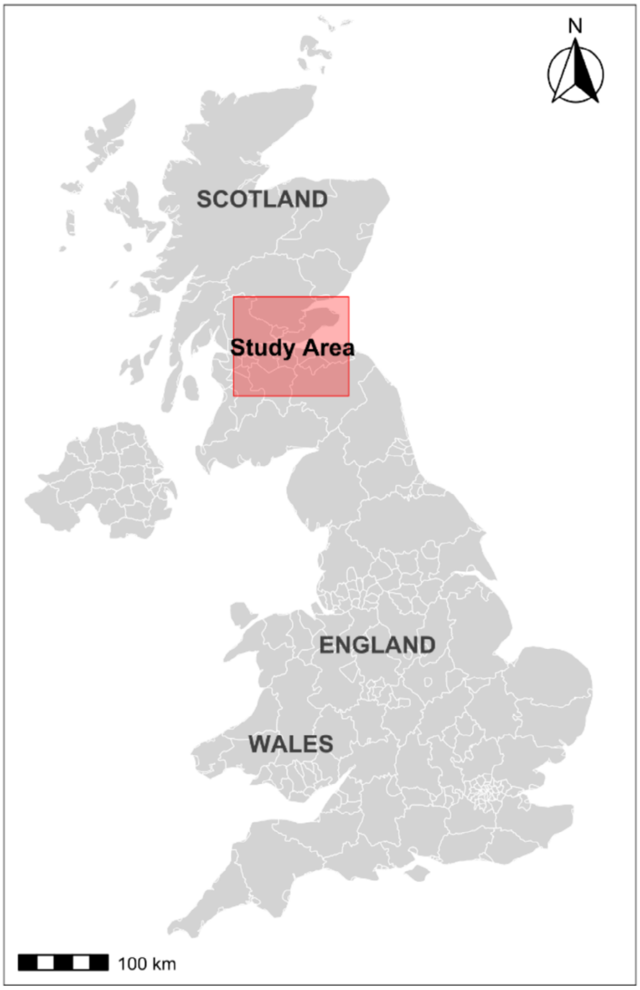

FIGURE 1 | Map showing the study area in central Scotland.

All woodland patches were established on land managed previously as agricultural land; specific management histories for individual sites prior to woodland establishment were not available. During the study period, woodland sites were subject only to minimal management, primarily periodic deer exclusion and, in some younger stands, low-level thinning. Pasture controls were managed as permanent grassland for grazing.

2.3 | Field Sampling Protocol

At each site, soils were sampled along a central transect, with points at least 10 m apart and > 1 m from tree stems (refer to Figure S1). For each site:

- Five cylindrical soil cores (15 cm × 4.5 cm) were taken per depth (0–15 cm, 15–30 cm) for aggregate fractionation.
- Five additional cores per depth were collected for bulk density and moisture.
- Ten auger samples per depth were obtained for elemental analysis (C, N) and pH.
- Organic layer material was sampled from three 0.25 m^2 quadrats at each site (except pastures where this horizon was absent).

All field protocols followed ICP Forests Pan-European guidelines (Cools and De Vos 2020). Samples were stored at 3.5°C until they were processed.

2.4 | Aggregate Fractionation and Carbon Analysis

Soil was air-dried, passed through an 8 mm sieve, and a 50 g subsample was used for wet-sieving aggregate fractionation into four classes: > 2000 μm, 2000–250 μm, 250–53 μm, and < 53 μm (following Six, Elliott, and Paustian 2000). Each class was oven-dried (40°C) and weighed. Sand correction was applied by dispersing a 5 g subsample in sodium hexametaphosphate, isolating sand retained on relevant sieves, and subtracting from aggregate mass.

After fractionation, aggregates and bulk soil were analysed for total C and N using a C:N elemental analyser. Soil organic carbon stocks ($t ha^{-1}$) were calculated using measured bulk density and % carbon. Soil pH was measured in a 1:2.5 soil:water suspension.

2.5 | Mean Weight Diameter

Stability interpretations were derived from aggregate size distributions by mathematically converting the proportional weight of the class aggregate into a simple index that represents the entirety of the sample, establishing the link between size distribution and stability. The soil Mean Weight Diameter was calculated as:

$$\text{MWD (mm)} = \sum_{i=1}^{n} \overline{x}_i w_i$$

where $\overline{x}_i$ is the mean diameter of fraction i (using 8 mm as the upper, and 0.025 mm as the lower boundary), and w_i is its sand-corrected proportion by mass.

2.6 | Statistical Analyses

2.6.1 | Data Structuring and Grouping

The woodland age gradient considered in this study reflects the developmental stages of temperate broadleaf woodland habitats as defined by Oliver and Larson (1996). To facilitate data analysis, the study sites were grouped into four age categories based on time since planting: stand initiation (≤30 years, $n=5$ sites), stem exclusion (31–80 years, $n=9$ sites), understory re-initiation (81–160 years, $n=16$ sites), and old growth sites (250+ years, $n=10$ sites). Pastures ($n=5$) functioned as an additional reference group. The discrepancy in sample sizes within these categories is due to the limited availability of soil types (namely Cambisols) that were the focus of our study.

2.6.2 | Soil Property Comparisons Across Land Uses

Non-parametric Kruskal–Wallis tests were used to compare bulk density, SOC, total C, soil moisture, and pH among land uses, followed by Dunn's tests for post hoc pairwise comparisons.

2.6.3 | Linear Mixed-Effects Modelling (LMM)

To analyse the effects of woodland age, depth, and aggregate fraction on soil properties, linear mixed-effects models (LMMs) were fitted using the 'lmer' function in R (R Core Team 2021) with Type III Wald chi-squared tests for fixed effects (Anova function, car package). Site ID was included as a random intercept to account for spatial clustering and repeated measures within sites.

2.6.4 | Model Specifications

- SOC stock analysis: SOC (t ha^{-1}) as response; fixed effects: woodland age category, depth; random effect: Site ID.
- Aggregate carbon concentration: Log-transformed total C concentration (g C kg^{-1}) as response; fixed effects: age category, aggregate size class, depth, and all two-way interactions (Age × Fraction, Age × Depth, Fraction × Depth); random effect: Site ID.
- Aggregate distribution: Proportion of water-stable aggregates (%) as the dependent variable with age and aggregate size as fixed effects.
- Aggregate carbon concentration: Log-transformed total C concentration (g C kg^{-1}) as response; fixed effects: age category, aggregate size class, depth, and their interactions; random effect: Site ID.
- Mean weight diameter: MWD (mm) as response; fixed effects: age category, depth, and Age × Depth interaction; random effect: Site ID.
- MWD as predictor of carbon: Log-transformed carbon concentration (g C kg^{-1}) and SOC stock (t ha^{-1}) as responses; fixed effects: MWD, depth; random effect: Site ID. Woodland age was excluded from these models to test MWD as an independent predictor.
- Post hoc pairwise comparisons were conducted using Tukey's Honest Significant Difference (HSD) test with $\alpha = 0.05$.

All continuous variables were assessed for normality and log-transformed where residuals deviated from model assumptions. Model simplification was guided by likelihood ratio tests and Akaike Information Criterion (AIC). Estimated marginal means (EMMs) post hoc comparisons were performed, with Tukey p-value adjustment for multiple testing.

2.6.5 | Effect Size and Variance Partitioning

Partial eta-squared (η^2) was calculated for fixed effects, and marginal and conditional R^2 values (variance explained by fixed and all effects, respectively) were reported for each model. Standardised effect sizes (Cohen's d) were computed for key pairwise contrasts. Effect sizes were interpreted following standard conventions: for partial η^2, values of 0.01, 0.06, and 0.14 represent small, medium, and large effects respectively (Cohen 1988); for Cohen's d, values of 0.2, 0.5, 0.8, and >1.0 represent small, medium, large, and very large effects.

All statistical analyses were performed in R v4.1.2, primarily using the lme4, emmeans, car, and effectsize packages.

3 | Results

3.1 | The Influence of Woodland Creation on Soil Carbon Stocks in Broadleaved Temperate Forests

Woodland soils showed substantially lower bulk density than pasture soils, with reductions of 0.22–0.28 g cm^{-3} representing a large effect ($\eta^2 = 0.25$, $p = 0.009$) across all woodland age categories (Table 1). No significant differences in bulk density were detected among woodland age categories, indicating that soil decompaction occurs rapidly following woodland establishment and is maintained throughout succession. Woodland soils were 1.0–1.5 pH units more acidic than pasture across both mineral soil depths, representing the strongest effect size among all measured soil properties ($\eta^2 = 0.24$, $p = 0.013$) and also demonstrated improved water retention capacity compared to pasture (moderate effect, $\eta^2 = 0.15$), though the effect of woodland age on soil moisture was not statistically significant ($p = 0.146$). Depth was a highly significant predictor of all three properties ($p < 0.001$ for bulk density, pH, and moisture; Table 1).

Woodland creation led to substantial increases in total SOC stocks (Figure 2), with mature woodlands (> 80 years) containing 88%–111% more carbon than pasture systems (71–80 vs. 38 t/ha, $p < 0.01$). Age effects were confined to the organic layer, where mature woodlands (> 80 years) contained significantly higher SOC than younger woodlands (≤ 80 years, $p < 0.01$). Post hoc comparisons showed that organic layer SOC stocks did not differ significantly between the ≤ 30 years and 31–80 years age groups ($p = 0.089$), indicating a modest but non-significant decline during the stem exclusion phase. No age-related differences were detected in mineral soil layers (0–15 cm and 15–30 cm, all $p > 0.05$).

The primary mechanism driving differences between woodland and pasture was organic layer development rather than mineral soil carbon accumulation, with the organic layer contributing 37%–42% of total woodland organic carbon stocks but being absent in pasture systems (Figure 2).

3.2 | The Influence of Woodland Age on Water-Stable Aggregates and Carbon Distribution

Woodland age had a large effect on aggregate carbon concentrations ($\eta^2 = 0.58$, $p < 0.001$). Aggregate size fraction ($\eta^2 = 0.25$, $p < 0.001$) and soil depth ($\eta^2 = 0.17$, $p < 0.001$) were also significant predictors. The Age × Depth interaction was significant ($\chi^2 = 9.78$, $p = 0.044$), indicating that age effects varied between soil layers. Together, the fixed effects explained 73% of total variance in log-transformed aggregate carbon concentrations, indicating strong predictive relationships.

The transition from pasture to ancient woodland represented major ecosystem changes across all aggregate fractions, with effect sizes ranging from medium to very large (Cohen's $d = 0.53$–1.28). The largest aggregates (> 2000 μm) showed the strongest response to woodland development ($d = 1.28$), representing more than one standard deviation increase in carbon concentration. Carbon

TABLE 1 | Mixed effects model ANOVA results for soil properties across woodland age categories and soil depths.

Response variable	Effect	χ^2	df	p	η^2	
Bulk density	Age range	13.55	4	0.009	0.253	**
Bulk density	Depth	35.03	1	< 0.001	0.04	***
pH (H_2O)	Age range	12.67	4	0.013	0.24	*
pH (H_2O)	Depth	35.96	1	< 0.001	0.041	***
Soil moisture	Age range	6.82	4	0.146	0.146	ns
Soil moisture	Depth	191.99	1	< 0.001	0.185	***

Note: Models included woodland age category and soil depth as fixed effects with site as a random effect to account for spatial clustering. η^2 = partial eta-squared representing the proportion of variance explained by age category effects. Significance levels: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns = non-significant.

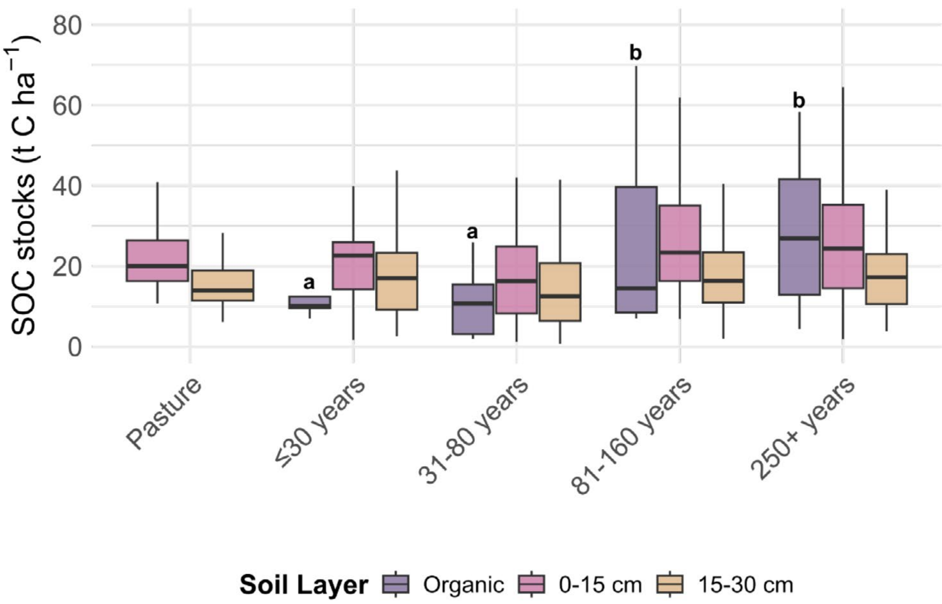

FIGURE 2 | Soil organic carbon stocks across woodland succession and soil layers. Letters indicate significant differences between age categories within the organic layer only (Kruskal–Wallis $p = 0.002$, post hoc with Tukey adjustment). Age effects on SOC stocks were tested for all layers: Significant in the organic layer ($p = 0.002$), but not in the 0–15 cm ($p = 0.089$) or 15–30 cm ($p = 0.059$) mineral soil layers. Outliers > 80 t C ha^{-1} not shown. The organic layer is absent in pasture systems. Boxes show median and interquartile range; whiskers extend to 1.5 × IQR.

concentrations consistently decreased from macroaggregates to microaggregates, with the largest fraction containing (> 2000 μm) containing the highest concentrations across all woodland ages (Figure 3). Pairwise comparisons of the land-use effect (pasture vs. ancient woodland) revealed large to very large effect sizes across all aggregate fractions: > 2000 μm (Cohen's $d = 1.28$, very large), 2000–250 μm ($d = 1.13$, large), < 53 μm ($d = 0.91$, large), and 250–53 μm ($d = 0.53$, medium). The strongest response in the largest aggregates supports the aggregate hierarchy model, whereby macroaggregates are formed around fresh organic inputs and are most sensitive to land-use change (Six, Paustian, et al. 2000; Tisdall and Oades 1982).

Macroaggregates (> 250 μm) consistently contained higher carbon concentrations than microaggregates (≤ 250 μm) across all age classes ($p < 0.001$). This difference was most pronounced in early succession (≤ 30 years) and ancient woodland (250+ years) sites.

Compared to pasture, the carbon in the 250–53 μm fraction was significantly higher in the 31–80 and the 81–160 age groups (approximately double; $p < 0.01$ for both comparisons). Woodlands showed elevated carbon concentrations in the < 53 μm silt and clay fraction, particularly at the 81–160 years (72% to 86% increase relative to pasture; $p = 0.003$, Cohen's $d = 0.91$). The depth effect was significant across all fractions ($\chi^2 = 26.06$, $p < 0.001$, $\eta^2 = 0.17$), with surface soils (0–15 cm) containing higher carbon concentrations than subsurface soils (15–30 cm). Notably, the 31–80-year woodland group displayed an even distribution of carbon among aggregate size classes across both mineral layers, suggesting a transitional phase of aggregate reorganisation during mid-succession.

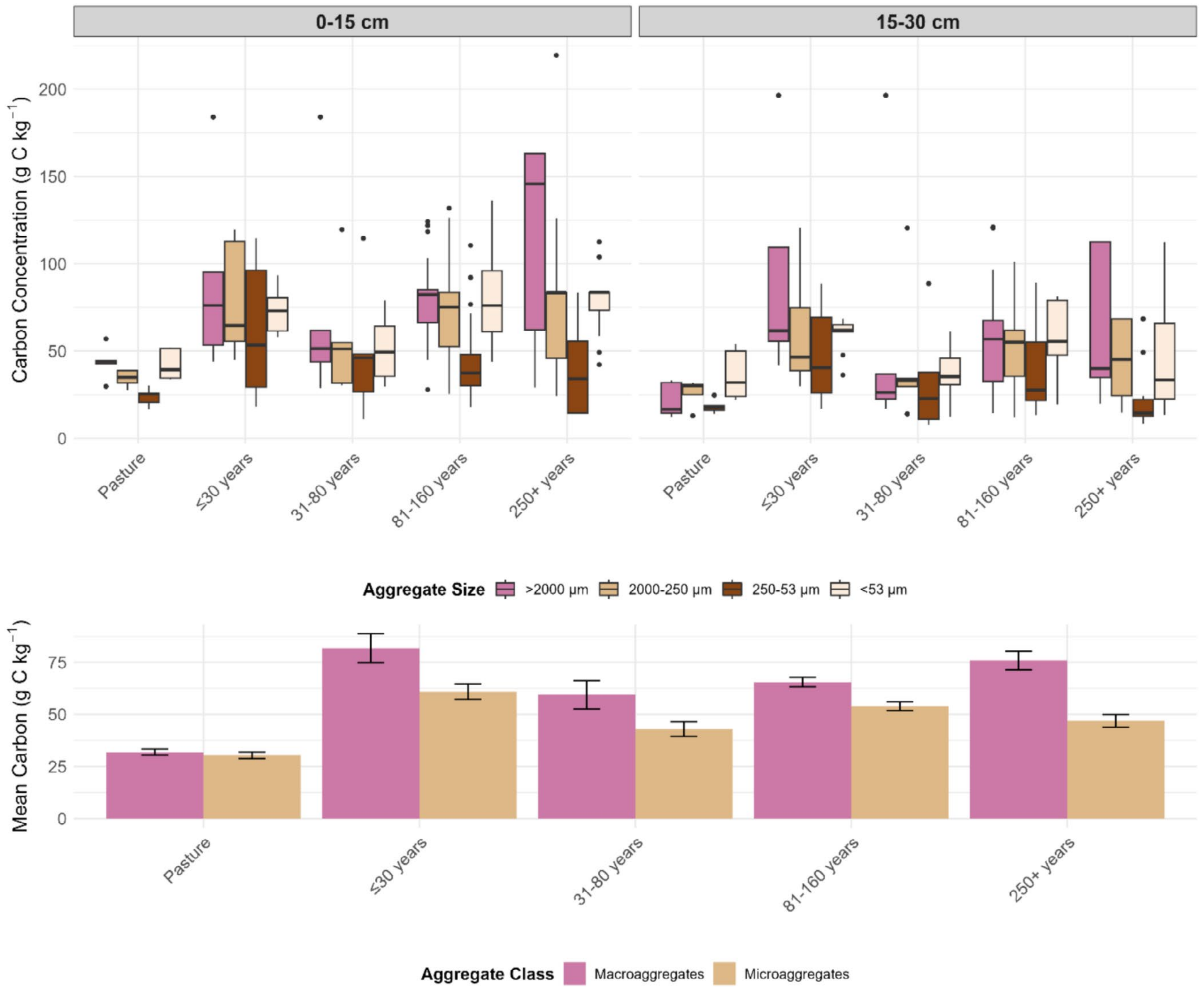

FIGURE 3 | Total carbon concentration in water-stable aggregates across woodland succession chronosequence. Top: Total carbon concentration ($g\,C\,kg^{-1}$) in different aggregate size fractions for 0–15 cm and 15–30 cm depth intervals. Bottom: Comparison of carbon concentrations between macroaggregates (> 250 μm, magenta) and microaggregates (≤ 250 μm, tan) across age classes. Box plots show median, quartiles, and outliers. Mixed model ANOVA showed significant effects of Age ($\chi^2 = 30.14$, $p < 0.001$, $\eta^2 = 0.58$), Fraction ($\chi^2 = 21.74$, $p < 0.001$, $\eta^2 = 0.25$), and Depth ($\chi^2 = 26.06$, $p < 0.001$, $\eta^2 = 0.17$). See Table S2 for full ANOVA results.

3.3 | Soil Stability Relationship With Woodland Age and Carbon Sequestration

Aggregate stability (MWD) was influenced by woodland age ($\chi^2 = 11.82$, $p = 0.019$, $\eta^2 = 0.17$), but this effect varied significantly with soil depth (Age × Depth interaction: $\chi^2 = 42.88$, $p < 0.001$). In the 0–15 cm layer, older secondary woodlands (81–160 years) showed significantly higher aggregate stability than pasture ($p = 0.020$, Tukey HSD), while ancient woodlands (250+ years) did not differ significantly from pasture ($p = 0.627$). No significant pairwise differences in aggregate stability were detected in the 15–30 cm layer (all $p > 0.15$), indicating that woodland effects on soil structure are confined to surface horizons (Figure 4).

MWD was not a statistically significant predictor of carbon concentration ($\chi^2 = 1.74$, $p = 0.188$, $\eta^2 = 0.002$) or SOC stocks ($\chi^2 = 0.99$, $p = 0.319$, $\eta^2 = 0.001$) in mineral soil layers (Table 2). These negligible effect sizes confirm that aggregate stability, as measured by MWD, does not reliably indicate carbon status in these systems. Soil depth, in contrast, was a strong predictor of both carbon concentration ($\eta^2 = 0.28$, $p < 0.001$) and SOC stocks ($\eta^2 = 0.08$, $p < 0.001$).

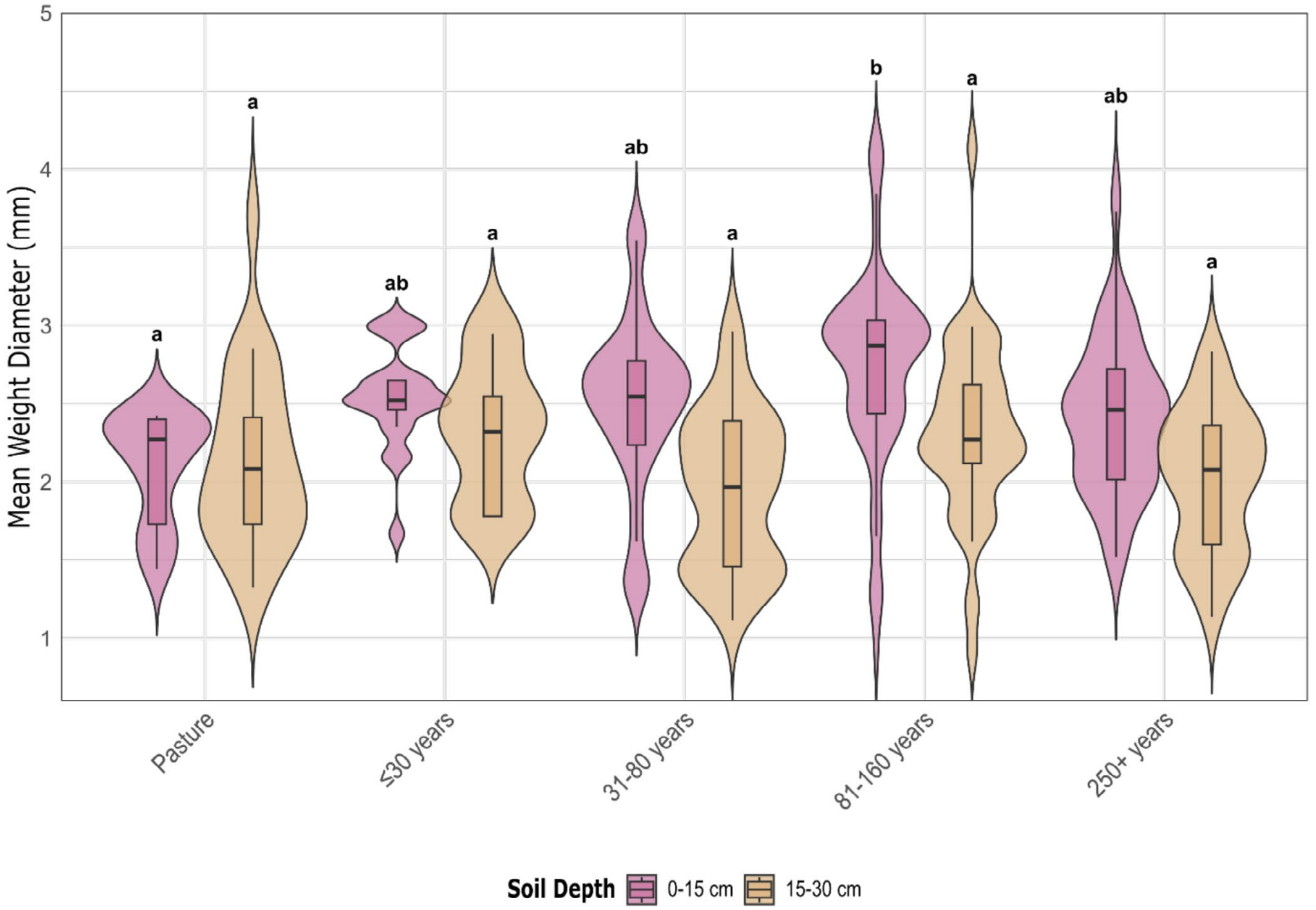

FIGURE 4 | Mean weight diameter (MWD) for the different age categories at two soil depths. Letters indicate significant differences within each depth (Tukey HSD, $p < 0.05$). The Age × Depth interaction was significant ($\chi^2 = 42.88$, $p < 0.001$), indicating age effects between depths. At 0–15 cm, pasture (a) differed significantly from 81 to 160-year woodlands (b); groups sharing letters (ab) did not differ significantly from either. At 15–30 cm, no significant pairwise differences were detected (all groups share letter 'a'). The violin plots show the probability density of the data; overlaid box plots display the median and interquartile range.

TABLE 2 | Analysis of variance results from mixed effects model testing MWD and soil depth as predictors of soil organic carbon stocks and total carbon concentration.

Response variable	Effect	χ^2	df	p	η^2	
Carbon concentration	MWD	1.74	1	0.188	0.002	ns
Carbon concentration	Depth	334.3	1	< 0.001	0.283	***
SOC stocks	MWD	0.99	1	0.319	0.001	ns
SOC stocks	Depth	71.49	1	< 0.001	0.077	***

Note: Models include both mineral soil depths (0–15 cm and 15–30 cm) with Depth as a fixed effect and Site as a random effect. Successional stage was excluded to test MWD as an independent predictor. Significance levels: ***$p < 0.001$, ns = non-significant.

4 | Discussion

4.1 | Woodland Creation and Carbon Stocks: Layer-Specific Mechanisms

Our results demonstrate that converting agricultural land to broadleaved woodland leads to substantial increases in soil organic carbon stocks — a finding in line with large-scale syntheses documenting SOC gains following woodland creation and rewilding of agricultural land (Guo and Gifford 2002; Paul et al. 2002). A key novel insight from this study, however, is the explicit quantification of these gains within the soil profile: *almost all the SOC increase was confined to the organic surface horizon*, which contributed up to 42% of total woodland SOC stocks and was largely absent in pastures. In contrast, mineral soil layers (0–15 and 15–30 cm) showed no consistent age-related or land-use differences in SOC stocks, even in the oldest woodlands. This layer-specific highlight clarifies a common gap in previous studies that often pool all soil horizons, overlooking the dominant influence of organic matter accumulation at the surface (Poeplau and Don 2013; Poeplau et al. 2024).

While prior work has shown that SOC accumulation is typically most rapid in the early decades post-afforestation — especially on former cropland (Paul et al. 2002; Laganière et al. 2010) — our data show that after initial woodland establishment, organic-layer development, rather than continuing mineral soil accrual, drives long-term increases in SOC. This finding echoes recent reviews emphasising the importance of litter and organic surface inputs (Guo and Gifford 2002; Laganière et al. 2010; Chenu et al. 2019), and suggests that management efforts to maximise SOC in reforested landscapes should prioritise protection and maintenance of the developing organic horizon.

4.2 | Successional Dynamics and Nonlinear SOC Trends

Carbon accumulation across the woodland chronosequence was not strictly linear. SOC stocks during the 31–80-year 'stem exclusion' stage compared to earlier (≤ 30 years) and later (81–160 years) stages (Figure 2), though this decline was not statistically significant (see Section 3), potentially due to greater allocation of plant carbon aboveground during this intensely competitive growth phase (Haynes and Gower 1995; Vicca et al. 2012). This dip is later reversed in older woodlands (> 80 years), where understory re-initiation and more complex vegetative structure likely increase both litter input and belowground carbon allocation (Oliver and Larson 1996). These results illustrate the importance of *considering successional stage — not age alone — when evaluating carbon trajectories* in regenerating forest ecosystems.

4.3 | Age-Dependent Shifts in Aggregate Carbon Pools and Soil Structure

Our study provides granular, *fraction-specific evidence for robust age-related changes* in the distribution and protection of SOC within soil aggregates. Carbon concentration within all water-stable aggregate fractions increased following woodland establishment, with effect sizes ranging from medium to very large. The largest effect observed in macroaggregates (> 2000 μm, Cohen's $d = 1.28$), consistent with the aggregate hierarchy model which predicts that macroaggregates, formed around fresh particulate organic matter, respond most rapidly to changes in organic inputs (Six, Paustian, et al. 2000). Notably, we found a relatively even distribution of SOC across aggregate classes in mid-aged woodlands (31–80 years), a pattern not observed in either younger or older woodlands. This may reflect a phase of dynamic soil structural reorganisation and microbial turnover, aligning with previous work that links aggregate heterogeneity to successional transition (Jastrow et al. 1998).

In older woodlands (81–160 years), the highest carbon increases were detected in microaggregates and the < 53 μm silt and clay fraction, suggesting progressive stabilisation of organic matter in finer soil domains over time. This is a process recognised as a hallmark of late-successional forests and critical for long-term carbon persistence (Six et al. 2002). These findings also reinforce field and meta-analytical work highlighting the importance of aggregation for enhancing SOC security (Lützow et al. 2006; Chenu et al. 2019).

Moreover, earthworm functional diversity, shown in other research to vary with woodland age (Ashwood et al. 2019), may help explain observed shifts in SOC distribution. Earthworms contribute to aggregate formation through the ingestion and excretion of soil particles, creation of biopores, and incorporation of organic matter into mineral soil (Six et al. 2004; Blouin et al. 2013). Anecic species, which become more abundant in older woodlands, are particularly effective at translocating surface litter into deeper soil layers and forming stable casts. Such mechanisms warrant deeper investigation in future chronosequence studies.

4.4 | Fine Fraction (< 53 μm): A Key Stabilisation Pool in Mature Woodlands

Although not a true aggregate class, the 53 μm silt and clay fraction was a consistent location of carbon enrichment across woodlands. In the 81–160 year group, *this fraction held 72% more carbon than in pasture soils*, revealing a long-term pathway for biochemical stabilisation through organo-mineral associations (Schmidt and Kögel-Knabner 2002; Lützow et al. 2006). This result asserts a growing consensus that silt and clay-associated C plays a disproportionately large role in long-term SOC persistence in forest systems (Atere et al. 2020).

4.5 | Aggregate Stability (MWD): Limited Indicator Value for SOC Monitoring

Improvements in aggregate stability (measured as MWD) were predominantly detected in the uppermost 0–15 cm mineral soil, where secondary woodlands aged 81–160 years displayed the strongest gains relative to pasture. However, *this effect did not persist into the deepest mineral layer nor with increasing woodland age into ancient stands*, suggesting a non-linear, possibly plateauing or oscillating trajectory of physical soil improvement through succession (Laganière et al. 2010; Fornara and

Tilman 2012). This surface-limited and non-linear response is rarely documented in detail and *underlines the need for vertical resolution in monitoring programs*.

Importantly, *MWD was not a statistically significant predictor of aggregate carbon content or total SOC stocks in our models* (Table 2): MWD explained only a negligible fraction of variance in either property, and correlations were weak regardless of woodland age.

This critical appraisal challenges the widespread practice of using MWD as a stand-alone indicator of soil C sequestration (Lehman et al. 2015; Chenu et al. 2019), indicating that physical aggregation often becomes decoupled from C storage in mature or late-successional forests, possibly due to the increasing importance of biochemical stabilisation mechanisms (Six et al. 2004).

4.6 | Implications for Soil Management and Carbon Accounting

Our findings highlight several points for managers and policy makers:

- *Enhancement and protection of the organic surface layer should be a priority* in woodland restoration strategies, given its outsized role in SOC accumulation.
- *Assessments of soil carbon benefits from woodland creation should explicitly include organic horizons* rather than rely solely on mineral soil sampling.
- *Reliance on physical metrics like MWD for SOC monitoring may be misleading*, especially in older or mature systems—carbon partitioning among aggregate size classes and layers provides better insight.
- *SOC gains in mineral soils may be limited*, especially when baseline conditions are already high in SOC, aligning with evidence that past land-use and initial soil properties modulate long-term sequestration outcomes.
- *Forest age and successional stage should be included in carbon models*, as SOC patterns vary markedly over time and depth.

5 | Conclusion

This study provides detailed mechanistic insight into how woodland age shapes aggregate carbon distribution and soil structure, challenging some common assumptions about soil carbon accrual and measurement after woodland creation. For effective evaluation of afforestation/reforestation outcomes and woodland soil management, future research and policy assessment should implement a layered, fraction-resolved framework. This approach must account for the vertical distribution of carbon, differences among aggregate size classes, and the successional development of the forest stand. Such precision will improve the quantification of woodland contributions to long-term soil carbon sequestration and enhance the scientific basis for soil health monitoring in forest establishment projects.

Acknowledgements

This study was made possible through generous financial support from a range of organisations, including Forest Research, University of Stirling, Forestry Commission, Natural England, DEFRA, The National Forest, Scottish Natural Heritage, and Tarmac. Our sincere thanks go to Big Stig and Dave Darroch for their invaluable assistance during fieldwork, as well as to Ian Washbourne and Francois Bouchereau for their contributions in the laboratory. We extend our gratitude to Kevin Watts, Franziska Steiner, Francois-Joly Xavier, and Johan Six for their insightful suggestions and meaningful discussions. We are also deeply appreciative of the landowners who kindly granted us access to their woodlands. Their cooperation was essential to the successful completion of this study.

Funding

This work was supported by Forest Research, UK. University of Stirling, Forestry Commission, Natural England, DEFRA, The National Forest, Scottish Natural Heritage and Tarmac.

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Abbas, F., Z. Zhu, and S. An. 2021. "Evaluating Aggregate Stability of Soils Under Different Plant Species in Ziwuling Mountain Area Using Three Renowned Methods." *Catena* 207: 105616. https://doi.org/10.1016/j.catena.2021.105616.

Ashwood, F., K. Watts, K. Park, E. Fuentes-Montemayor, S. Benham, and E. I. Vanguelova. 2019. "Woodland Restoration on Agricultural Land: Long-Term Impacts on Soil Quality." *Restoration Ecology* 27: 13003. https://doi.org/10.1111/rec.13003.

Atere, C. T., A. Gunina, Z. Zhu, et al. 2020. "Organic Matter Stabilization in Aggregates and Density Fractions in Paddy Soil Depending on Long-Term Fertilization: Tracing of Pathways by ^{13}C Natural Abundance." *Soil Biology and Biochemistry* 149: 107931. https://doi.org/10.1016/j.soilbio.2020.107931.

Blouin, M., M. E. Hodson, E. A. Delgado, et al. 2013. "A Review of Earthworm Impact on Soil Function and Ecosystem Services." *European Journal of Soil Science* 64: 161–182. https://doi.org/10.1111/ejss.12025.

Bougma, A. B., K. Ouattara, H. Compaore, et al. 2022. "Soil Aggregate Stability of Forest Islands and Adjacent Ecosystems in West Africa." *Plant and Soil* 473, no. 1: 533–546. https://doi.org/10.1007/s11104-022-05302-x.

Bronick, C. J., and R. Lal. 2005. "Soil Structure and Management: A Review." *Geoderma* 124, no. 1: 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005.

Chenu, C., D. A. Angers, P. Barré, D. Derrien, D. Arrouays, and J. Balesdent. 2019. "Increasing Organic Stocks in Agricultural Soils: Knowledge Gaps and Potential Innovations." *Soil and Tillage Research* 188: 41–52. https://doi.org/10.1016/j.still.2018.04.011.

Climate Change Committee. 2017. *Draft Climate Change Plan: Draft Third Report on Policies and Proposals 2017–2032*. Scottish Government.

Accessed April 8, 2023. http://www.gov.scot/publications/draft-climate-change-plan-draft-third-report-policies-proposals-2017/.

Cohen, J. 1988. *The Effect Size. Statistical Power Analysis for the Behavioral Sciences*, 77–83. Routledge.

Cools, N., and B. De Vos. 2020. "Part X: Sampling and Analysis of Soil. Version 2020-1." In *Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests*, edited by UNECE ICP Forests Programme Co-Ordinating Centre. Thünen Institute of Forest Ecosystems, ICP Forests. Accessed September 19, 2023. https://www.zotero.org/picoas66/search/icp/titleCreatorYear/items/QVHDHW7R/item-list.

de Sinquini Souza, R., I. Sousa Morais, J. S. Rosset, T. de Melo Rodrigues, A. Loss, and M. G. Pereira. 2024. "Aggregation as Soil Quality Indicator in Areas Under Different Uses and Managements." *Farming System* 2, no. 2: 100082. https://doi.org/10.1016/j.farsys.2024.100082.

DEFRA. 2022. *Woodland Cover Targets – Detailed Evidence Report*. Department for Environment Food and Rural Affairs.

FAO. 2010. *Global Forest Resources Assessment 2010: Main Report. FAO Forestry Paper 163*. FAO. http://www.fao.org/docrep/013/i1757e/i1757e.pdf.

FAO. 2015. *Status of the World's Soil Resources: Main Report*. FAO. Accessed April 8, 2023. https://www.fao.org/documents/card/en/c/c6814873-efc3-41db-b7d3-2081a10ede50/.

FAO. 2020. *Global Forest Resources Assessment 2020 – Key Findings, Global Forest Resources Assessment 2020*. FAO. https://doi.org/10.4060/CA8753EN.

Fornara, D. A., and D. Tilman. 2012. "Soil Carbon Sequestration in Prairie Grasslands Increased by Chronic Nitrogen Addition." *Ecology* 93, no. 9: 2030–2036. https://doi.org/10.1890/12-0292.1.

Fuentes-Montemayor, E., M. Ferryman, K. Watts, et al. 2020. "Small Mammal Responses to Long-Term Large-Scale Woodland Creation: The Influence of Local and Landscape-Level Attributes." *Ecological Applications* 30, no. 2: e02028. https://doi.org/10.1002/eap.2028.

Guo, L. B., and R. Gifford. 2002. "Soil Carbon Stocks and Land Use Change: A Meta Analysis." *Global Change Biology* 8: 345–360. https://doi.org/10.1046/j.1354-1013.2002.00486.x.

Haynes, B. E., and S. T. Gower. 1995. "Belowground Carbon Allocation in Unfertilized and Fertilized Red Pine Plantations in Northern Wisconsin." *Tree Physiology* 15, no. 5: 317–325. https://doi.org/10.1093/treephys/15.5.317.

Jackson, R. B., K. Lajtha, S. E. Crow, G. Hugelius, M. G. Kramer, and G. Piñeiro. 2017. "The Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls." *Annual Review of Ecology, Evolution, and Systematics* 48, no. 1: 419–445. https://doi.org/10.1146/annurev-ecolsys-112414-054234.

Jastrow, J. D., R. M. Miller, and J. Lussenhop. 1998. "Contributions of Interacting Biological Mechanisms to Soil Aggregate Stabilization in Restored Prairie." *Soil Biology and Biochemistry* 30, no. 7: 905–916. https://doi.org/10.1016/S0038-0717(97)00207-1.

Laganière, J., D. A. Angers, and D. Paré. 2010. "Carbon Accumulation in Agricultural Soils After Afforestation: A Meta-Analysis." *Global Change Biology* 16, no. 1: 439–453. https://doi.org/10.1111/j.1365-2486.2009.01930.x.

Lehman, R., R. M. Lehman, V. Acosta-Martinez, et al. 2015. "Soil Biology for Resilient, Healthy Soil." *Journal of Soil and Water Conservation* 70: 12–18. https://doi.org/10.2489/jswc.70.1.12A.

Lehmann, J., and M. Kleber. 2015. "The Contentious Nature of Soil Organic Matter." *Nature* 528, no. 7580: 60–68. https://doi.org/10.1038/nature16069.

Lützow, M. V., I. Kögel-Knabner, K. Ekschmitt, et al. 2006. "Stabilization of Organic Matter in Temperate Soils: Mechanisms and Their Relevance Under Different Soil Conditions – A Review." *European Journal of Soil Science* 57, no. 4: 426–445. https://doi.org/10.1111/J.1365-2389.2006.00809.X.

Maria Sabatini, F., S. Burrascano, W. S. Keeton, et al. 2018. "Where Are Europe's Last Primary Forests?" *Diversity and Distributions* 24: 20. https://doi.org/10.1111/ddi.12778.

Nave, L., E. Marín-Spiotta, T. Ontl, M. Peters, and C. Swanston. 2019. "Soil Carbon Management." In *Developments in Soil Science*, vol. 36, 215–257. Elsevier. https://doi.org/10.1016/B978-0-444-63998-1.00011-2.

Oliver, C. D., and B. A. Larson. 1996. *Forest Stand Dynamics*. FES Publications. https://elischolar.library.yale.edu/fes_pubs/1.

Ortiz-Oñate, C., M. J. Fernández-Alonso, B. Kitzler, E. Díaz-Pines, A. Rubio, and M. Benito. 2022. "Variations in Soil Aggregation, Microbial Community Structure and Soil Organic Matter Cycling Associated to Long-Term Afforestation and Woody Encroachment in a Mediterranean Alpine Ecotone." *Geoderma* 405: 115450.

Paul, K. I., P. J. Polglase, J. G. Nyakuengama, and P. K. Khanna. 2002. "Change in Soil Carbon Following Afforestation." *Forest Ecology and Management* 168, no. 1: 241–257. https://doi.org/10.1016/S0378-1127(01)00740-X.

Poeplau, C., and A. Don. 2013. "Sensitivity of Soil Organic Carbon Stocks and Fractions to Different Land-Use Changes Across Europe." *Geoderma* 192: 189–201. https://doi.org/10.1016/j.geoderma.2012.08.003.

Poeplau, C., T. Riefling, M. Schiedung, and R. Anlauf. 2024. "Land Use and Soil Property Effects on Aggregate Stability Assessed by Three Different Slaking Methods." *European Journal of Soil Science* 75, no. 4: e13549. https://doi.org/10.1111/ejss.13549.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Schmidt, M. W. I., and I. Kögel-Knabner. 2002. "Organic Matter in Particle-Size Fractions From A and B Horizons of a Haplic Alisol." *European Journal of Soil Science* 53, no. 3: 383–391. https://doi.org/10.1046/j.1365-2389.2002.00460.x.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. "A History of Research on the Link Between (Micro)aggregates, Soil Biota, and Soil Organic Matter Dynamics." *Soil and Tillage Research* 79, no. 1: 7–31. https://doi.org/10.1016/J.STILL.2004.03.008.

Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002. "Stabilization Mechanisms of Soil Organic Matter: Implications for C-Saturation of Soils." *Plant and Soil* 241: 155–176.

Six, J., E. T. Elliott, and K. Paustian. 2000. "Soil Macroaggregate Turnover and Microaggregate Formation: A Mechanism for C Sequestration Under No-Tillage Agriculture." *Soil Biology and Biochemistry* 32, no. 14: 2099–2103. https://doi.org/10.1016/S0038-0717(00)00179-6.

Six, J., K. Paustian, E. T. Elliott, and C. Combrink. 2000. "Soil Structure and Organic Matter: I. Distribution of Aggregate-Size Classes and Aggregate-Associated Carbon." *Soil Science Society of America Journal* 64: 681–689.

Tisdall, J., and J. Oades. 1982. "Organic Matter and Water-Stable Aggregates in Soils." *Journal of Soil Science* 33: 141–163.

UK Parliament. 2021. "Woodland Creation." POST Note Number 636. January 2021. London, UK.

van Bavel, C. H. M. 1950. "Mean Weight-Diameter of Soil Aggregates as a Statistical Index of Aggregation." *Soil Science Society of America Journal* 14: 20–23. https://doi.org/10.2136/sssaj1950.036159950014000C0005x.

van der Werf, G. R., D. C. Morton, R. S. DeFries, et al. 2009. "CO_2 Emissions From Forest Loss." *Nature Geoscience* 2, no. 11: 737–738. https://doi.org/10.1038/ngeo671.

Vanguelova, E. I., T. R. Nisbet, A. J. Moffat, S. Broadmeadow, T. G. M. Sanders, and J. I. L. Morison. 2013. "A New Evaluation of Carbon Stocks in British Forest Soils." *Soil Use and Management* 29, no. 2: 169–181. https://doi.org/10.1111/sum.12025.

Vicca, S., S. Luyssaert, J. Peñuelas, et al. 2012. "Fertile Forests Produce Biomass More Efficiently." *Ecology Letters* 15, no. 6: 520–526. https://doi.org/10.1111/j.1461-0248.2012.01775.x.

Watts, K. 2006. "British Forest Landscapes – The Legacy of Woodland Fragmentation." *Quarterly Journal of Forestry* 100: 273–279.

Watts, K., E. Fuentes-Montemayor, N. A. Macgregor, et al. 2016. "Using Historical Woodland Creation to Construct a Long-Term, Large-Scale Natural Experiment: The WrEN Project." *Ecology and Evolution* 6, no. 9: 3012–3025. https://doi.org/10.1002/ece3.2066.

Xiao, L., G. Liu, P. Li, S. Xue, and C. Zhang. 2023. "Carbon Stabilization Pathways in Soil Aggregates During Long-Term Forest Succession: Implications From $\delta^{13}C$ Signatures." *Soil Biology & Biochemistry* 177: 108900.

Zhou, M., C. Liu, J. Wang, et al. 2020. "Soil Aggregates Stability and Storage of Soil Organic Carbon Respond to Cropping Systems on Black Soils of Northeast China." *Scientific Reports* 10, no. 1: 1–13. https://doi.org/10.1038/s41598-019-57193-1.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** sum70162-sup-0001-DataS1.docx.