Estimating maximum fine fraction organic carbon in UK grasslands

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Abstract. Soil organic carbon (SOC) sequestration across agroecosystems worldwide can contribute to mitigate the effects of climate change by reducing levels of atmospheric CO_2. Stabilisation of organic carbon (OC) in the fine soil fraction (< 20 μm) is considered an important long-term store of SOC and the saturation deficit (difference between measured OC and estimated maximum OC in the fine fraction) is frequently used to assess SOC sequestration potential following the linear regression equation developed by Hassink, (1997). However, this approach is often taken without any assessment of the fit of the equation to the soils being studied. The statistical limitations of linear regression have previously been noted, giving rise to the proposed use of boundary line analysis and quantile regression to provide more robust estimates of maximum SOC stabilisation. The objectives of this work were to assess the suitability of the Hassink, (1997) equation to estimate maximum fine fraction OC in UK grassland soils of varying sward ages and to evaluate the linear regression, boundary line and quantile regression methods to estimate maximum fine fraction OC. A chronosequence of 10 grasslands was sampled, in order to assess the relationship between sward age (time since last reseeding event) and the measured and predicted maximum fine fraction OC. Significantly different regression equations show that the Hassink, (1997) equation does not accurately reflect maximum fine fraction OC in UK grasslands when determined using the proportion of fine soil fraction (< 20 μm, %) and measured fine fraction OC (g C kg⁻¹ soil). The QR estimate of maximum SOC stabilisation was almost double that of linear regression and BL analysis (0.89 ± 0.074, 0.43 ± 0.017 and 0.57 ± 0.052 g C kg⁻¹ soil, respectively). Sward age had an inconsistent effect on the measured variables and potential maximum fine fraction OC. Fine fraction OC across the grasslands made up 4.5 to 55.9% of total SOC, implying that there may be either high potential for additional C sequestration in the fine fraction of these soils, or protection in aggregates is predominant in these grassland soils. This work highlights the need to ensure that methods used to predict maximum fine fraction OC reflect the soil *in situ*, resulting in more accurate assessments of carbon sequestration potential.

1. Introduction

Carbon (C) sequestration in soils offers a significant opportunity to remove CO_2 from the atmosphere and store it into long lived C pools (Lal, 2004; Powlson et al., 2011), with co-benefits for soil structure and functioning (Lorenz and Lal, 2018; Smith, 2012; Soussana et al., 2004). To utilise soils as a CO_2 drawdown mechanism, accurate estimates of their storage capability are required. With respect to soil organic carbon (SOC) sequestration, organic carbon (OC) stabilised via adsorption to mineral surfaces in the fine soil fraction (< 20 µm) is often regarded as the most important due to its longer residence time (Baldock and Skjemstad, 2000; Six et al., 2002). There is empirical evidence that there is an upper protective capacity limit, or saturation point of the mineral stabilised OC pool (Six et al., 2002; Stewart et al., 2007). Potential SOC sequestration (or saturation deficit) can be estimated by subtracting the current fine fraction OC from the estimated maximum fine fraction OC (Angers et al., 2011).

Hassink, (1997) compared pairs of Dutch arable and grassland soils and found that while soil bulk SOC contents significantly differed among soils, fine fraction OC did not. These findings led to the idea that the saturation point of the fine soil fraction could be estimated by linear regression using the mass proportion of fine fraction in a soil sample (%) and the current fine fraction OC (g C kg^{-1} soil). Several iterations of the concept have been proposed to overcome the limitations of linear regression. For example, boundary line analysis uses a defined upper or lower subset of a data set to estimate the boundary line, when a limiting response to an independent variable(s) along a boundary is supported (Lark and Milne, 2016; Schmidt et al., 2000). Using the upper 90th percentile of a data set, boundary line analysis overcomes the limitation of linear regression depicting the mean response to the independent variable (Feng et al., 2013; Shatar and Mcbratney, 2004), which is thought to cause an underestimation of sequestration potential. Quantile regression estimates the response of a specific quartile using the entire data set. It also makes no assumptions regarding homogeneity of variance, thus increasing the robustness of the estimated maximum fine fraction OC. In quantile regression sample size is not reduced as in boundary line analysis (Beare et al., 2014; Cade and Noon, 2003). Using a forced zero intercept overcomes the contradiction of a positive intercept indicating the presence of fine fraction OC without any fine soil fraction (Beare et al., 2014; Feng et al., 2013; Liang et al., 2009). To our knowledge no comparisons between the equation developed by Hassink, (1997) and one generated in the same way with a different data set has been done within the UK. This type of assessment would help to determine the suitability of the Hassink, (1997) linear regression equation to predict maximum fine fraction OC in UK soils. Without this carbon sequestration potentials may be both over and underestimated. In the UK, human-managed grasslands are the dominant land use, covering 36% of the land area (Ward et al., 2016). Managed grasslands are planted and maintained to increase agricultural productivity through fertiliser and liming applications, and the re-seeding of swards. The high levels of disturbance associated with re-seeding events by mould board ploughing and harrowing in particular, result in changes in soil structure, , nutrient cycling and SOC mineralisation (Carolan and Fornara, 2016; Drewer et al., 2017; Soussana et al., 2004). Organo-mineral associations form the basis of microaggregates (Baldock and Skjemstad, 2000). The destruction of aggregates makes the organic carbon protected

65   within the aggregates more accessible for mineralisation by the soil microbial community. This may result in the increased mineralisation of existing SOC, known as the priming effect (Kuzyakov et al., 2000) The long-term effect of such re-seeding event on SOC dynamics is understudied, it is therefore important to understand how disturbance might affect OC in the fine fraction, and the SOC sequestration ability of managed grasslands.

70   The objectives of this study were (i) to assess the suitability of the Hassink, (1997) equation to estimate maximum fine fraction OC in UK grassland soils, (ii) to evaluate the linear regression, boundary line and quantile regression methods to estimate maximum fine fraction OC, and (iii) to explore the relationship between sward age (time since last reseeding event), and current and predicted maximum fine fraction OC. We hypothesised that (i) the linear regression equation developed using UK grassland soils would be significantly different to that of Hassink, (1997), and that (ii) grasslands with an older sward age,

75   would have a greater proportion of total SOC stabilised in fine fraction (< 20 μm) and a lower sequestration potential.

2. Materials and Methods

2.1 Site Description and Sampling

Ten grassland chronosequences covering a wide range of soil types, land use and climatic conditions were identified across the UK in 2016. The sites included the range of agricultural activity associated with UK grasslands (upland grazing, dairy, and

80   mixed grazing), variations in soil type (organo-mineral, mineral and chalk) and the majority of UK climatic zones (Table 1). At each location, five to eight individual fields of different sward age (represented by years since a ploughing and reseeding event), ranging from 1 to 179 years, were identified for sampling. In each field, areas were avoided which had different applications of manure, soil types or topography, headlands, areas near gates, where lime or manure had previously been dumped, or where livestock congregate. Two replicate soil cores were collected to a depth of 30 cm using a soil auger with a

85   2.5 cm diameter steel core and bulked to give a single composite sample. This was repeated 10 times in each field at regular intervals in a 'W' shape across the field totalling 10 replicate samples per field per site. Intact soil cores for determining bulk densities were collected at three locations in each field at two depths (10 to 15 cm and 20 to 25 cm) using intact rings (7.5 cm diameter, 5 cm height). Replicate samples were sieved to 2 mm and fresh subsamples were used to determine soil pH in water. Remaining sieved soils were dried at 40°C and ball milled prior to determination of total C and N contents (% by mass) using

90   a Flash 2000 elemental analyser. Intact soils were dried at 107°C and weighed to calculate dry bulk densities. Any stones were removed.

2.2 Soil fractionation

The fine fraction (< 20 μm) of the soil was separated using a combined ultrasonic dispersion and sedimentation method adapted from Hassink, (1997). Briefly, 20 g of dried sieved soil was soaked in 100 mL of deionised water for 24 hours. The suspension was then sonicated with a Microson XL2000 Ultrasonicator for 20 minutes at 20 W in 50 mL centrifuge tubes, surrounded by ice to prevent overheating. The separated samples were recombined in 150 mL tubes, and shaken end over end to disperse the soil water suspension. Sedimentation times were determined using a table applying Stokes Law, for 20 μm particles, a particle density of 2.65 g cm^{-3} and sedimentation depth of 5 cm at temperatures between 20ºC and 35ºC (Jackson, 2005). After the appropriate sedimentation time, the fine fraction was siphoned off the soil suspension. The fine fraction was dried for 24 hours at 107°C and ball milled prior to total C and N analysis (% by mass) using a Flash 2000 Elemental Analyser, to determine the current OC content of the fine fraction. At each site, a minimum of 3 fields varying in age (young, intermediate, and old at that location) were selected, and 3 of the 10 replicate field samples were selected at random for fractionation.

Hydrochloric acid (HCl) fumigation was used to remove carbonates from the Plumpton samples. Ball-milled samples, in silver capsules, were moistened with deionised water (1:4 sample: water ratio) to aid the efficiency of carbonate removal by HCl fumes (Dhillon et al., 2015). The samples were placed in a vacuum desiccator with a beaker of 100 mL of 12 M HCl, for 24 hours and subsequently dried in a ventilated oven at 60°C for 16 hours, to remove excess moisture and HCl (Dhillon et al., 2015). Total C and N contents were determined as outlined above.

2.3 Statistical analyses

All statistical analyses were carried out using R software version 3.5.3 (Team, 2019). Significant differences were determined by ANOVA's and by post-hoc Tukey tests ($\alpha = 0.05$). Where assumptions of normality and variance were not satisfied by testing (Shapiro Wilkens and Levenes Test) significant differences were identified using Kruskal test and post hoc Dunn test. A Kendal tau (τ) correlation matrix was produced using the 'corrplot' package (Wei and Simko, 2017).

2.3.1 Regression analyses

Linear regression was used to predict maximum fine fraction OC, with the mass proportion of fine fraction (< 20 μm, %) in a sample and the measured OC of the fine fraction (g C kg^{-1} soil) as the independent and dependent variables, respectively. Regression equations were developed for the combined UK data set, and the individual sites. Linear regression with a forced zero intercept was used with data from this study and the data published in Hassink (1997).

Boundary line analyses were performed as an alternative to linear regression, both with and without a forced zero intercept to predict maximum fine fraction OC for all UK sites. The data was organised by mass proportion of the fine fraction

(%) and divided into subgroups at 5, 10 and 15% intervals. The 10% interval reflects the method of Feng et al. (2013), whilst the 5 and 15% intervals were used to assess the effect of interval on estimation of maximum fine fraction OC. The groups were then ordered by measured fine fraction OC (g C kg^{-1} soil), and the values in the 90th percentile were used to plot the boundary line. Boundary line analysis was not used for individual sites as it resulted in too few data points. Quantile regression analysis was performed in Rstudio using the 'quantreg' package (Koenker, 2019), for the 90th and median percentiles ($\tau = 0.90$ and $\tau = 0.50$). Forcing the intercept to zero overcomes the paradox of having C stabilised as MAOC without any fine fraction in the soil. Significant differences between slopes were identified using the 'lsmeans' package (Lenth, 2016), followed by post-hoc Tukey tests ($\alpha = 0.05$).

2.3.2 Carbon saturation ratio

The carbon saturation ratio was determined to identify the degree of saturation across the sites. The carbon saturation ratio was calculated by dividing the current fine fraction OC by the estimated maximum fine fraction OC content. Values < 1 were deemed under saturated, = 1 as at saturation and > 1 as oversaturated.

3. Results

3.1 Current C concentrations

The measured total SOC and fine fraction OC concentrations varied within the grassland sites (Fig. 1). Total SOC ranged from 8.2 to 85.8g C kg^{-1} soil, with a median of 32.7 g C kg^{-1} soil. Hillsborough, Overton and Plumpton had significantly higher total SOC, whilst Harpenden and Llangorse had the lowest total SOC ($P < 0.05$) (Fig. 1). The measured fine fraction OC ranged from 1.4 to 20.9 g C kg^{-1} soil, with a median of 6.2 g C kg^{-1} soil. Overton had the highest total fine fraction OC ($P < 0.05$) and was the only organically managed site (Fig. 1). The proportion of OC stabilised in the fine fraction (< 20 μm) had high variability across the UK sites accounting for 4.5 to 50.1% of total SOC with a median of 17.5%. The proportion of total SOC stabilised in the fine fraction (< 20 μm), and proportion of fine fraction in a sample did not significantly differ in Harpenden and Overton. However they have significantly different measured fine fraction OC contents (g C kg^{-1} soil) ($P < 0.05$), indicating different saturation potentials (Fig. 1). Soil C: N ratio was positively correlated with fine fraction C: N (0.30, P < 0.0001), Table 2, however there was no relationship between bulk soil C: N ratio and proportion of fine fraction (data not shown). The fine fraction and bulk soil C: N ratios were significantly different between the sites, Figure 1. The mean value of fine fraction showed little deviation, 9.84 ± 1.00 (mean ± standard deviation). Full details of all the measured properties of bulk and fine fraction, per field are presented in Table A1.

The significance of correlations between the measured soil properties, time since reseeding and known environmental factors were analysed. The matrix of Kendall tau (τ) correlation coefficients in Table 2, revealed that measured fine fraction OC was positively correlated with median annual temperature ($\tau = 0.13$, $P < 0.05$), %N ($\tau = 0.26$, $P < 0.0001$) and %C ($\tau = 0.27$, $P < 0.0001$) in the bulk soil, and negatively correlated with mean annual rainfall ($\tau = -0.36$, $P < 0.0001$), and %N ($\tau = -0.15$, $P < 0.05$) in the fine fraction. Mass proportion of fine fraction and measured fine fraction OC (g C kg^{-1} soil) were positively correlated in cambisols ($R^2 = 0.61$, $P < 0.05$), gleysols ($R^2 = 0.76$, $P < 0.05$), podzols ($R^2 = 0.93$, $P < 0.05$), and stagnosols ($R^2 = 0.88$, $P < 0.05$) (Fig. 2). However, the proportion of total SOC stabilised in the fine fraction (< 20 μm), was greatest in luvisols ($P < 0.05$) (Fig. 3).

3.2 Estimated maximum fine fraction organic carbon

The slope generated from the UK data used to estimate maximum fine fraction OC (Table 3) was significantly different ($P < 0.05$) to the slope reported in Hassink (1997). There was no significant difference between the slopes generated from the UK data and the data from Hassink (1997), when estimated by linear regression with a forced zero intercept. Significantly, different ($P < 0.05$) slopes were found between the individual UK sites, owing to the range in the proportion of the fine fraction within each sample, from 1.85 to 51.8%, (Tables A3 and A4).

Coefficients from boundary line analysis are presented in (Table 3). There was no significant difference in slopes between the 5, 10, and 15% fine fraction intervals used. The median percentile quantile regression analysis had a similar slope to the boundary line and linear regression with forced zero intercept. Quantile regression using the 90th percentile resulted in the steepest slope of all estimation methods (Table 3). The C saturation ratios revealed the difference in number of samples with potential to sequester more C (Table 4). The Hassink (1997) linear regression equation, without a forced zero intercept, predicted the greatest number of unsaturated sites, followed by the 90th percentile quantile regression, with a forced zero intercept. There was no clear relationship between oversaturated sites and proportion of silt and clay contents as oversaturation occurred across all proportions, indicated by points above the lines in Fig. 4.

3.3 Effect of sward age on current C concentrations and estimated maximum MAOC

Sward age (years since last reseeding event) had a weak positive correlation with the mass proportion of the fine fraction (%) (Table 2). When grouped in five year intervals, significant differences were found between age group and the mass proportion of the fine fraction (%), measured fine fraction OC (< 20 μm), (g C kg^{-1} soil), and the C:N ratio of the fine fraction (Table 5), however there was no consistent increase or decrease with sward age. At each site, significant differences were observed between fields, for some properties, but again there was no consistent effect of sward age (Tables A3 and A4).

4. Discussion

4.1 Estimation of maximum fine fraction organic carbon

Determining the potential C sequestration capacity of soils is essential to predict the influence of land management for climate change mitigation. The determination of saturation deficit using the mass proportion of the fine fraction and current fine
185 fraction OC content is an established method with a strong grounding in correlation between the variables. As mentioned earlier, previous studies have examined methods to improve estimates of maximum fine fraction OC. However so far no comparison has been made between the Hassink, (1997) linear regression equation, and one developed using grassland soils in the UK.

The significantly different slopes for the linear regression equations (Table 3) shows that the Hassink (1997)
190 regression equation is not suitable for estimating maximum fine fraction OC in UK grasslands. Previous concerns have focused on the potential for the equation developed by Hassink (1997) to underestimate maximum fine fraction OC, as least squares linear regression represents the mean response of the independent variable, rather than the maximum. For the UK grasslands in this study, estimating maximum fine fraction OC using the Hassink (1997) regression approach resulted in a significant overestimation of fine fraction OC sequestration potential. Future work using maximum fine fraction OC predictions equations
195 reported in the literature (e.g. Beare et al., 2014; Feng et al., 2014; Hassink, 1997; Six et al., 2002) should first conduct a validity test, and determine if the regression equations match the soils in question or a subset of the data, to ensure results are not significantly over or underestimated.

To overcome the contradiction of an intercept greater than zero, indicating that C is stabilised in the fine fraction without any fine fraction, a forced zero intercept was used. The linear regression slopes with a forced zero intercept were not
200 significantly different, and were similar to that of Feng et al. (2013), 0.42 ± 0.002. Liang et al. (2009) reported a lower slope of 0.36 in Chinese black soils, whilst Beare et al. (2014) reported a slope of 0.70 ± 0.03 in long-term New Zealand pastures. The range of reported values, and differences across the UK sites (Tables A3 and A4), suggest that the effect of the proportion of fine fraction of a sample on fine fraction OC is not consistent and likely reflects differences in pedogenic and environmental conditions, land management and possibly the fine fraction OC isolation method. It may be that the use of the mass proportion
205 of fine fraction to predict maximum fine fraction OC is only suited on larger scales, rather than smaller, site specific scales, as indicated by the variability in this study.

Boundary line analysis and quantile regression have been suggested as alternatives to overcome the limitations of linear regression. The estimation of maximum fine fraction OC was greatest when using quantile regression ($\tau = 0.90$), whereas boundary line estimates at 5 and 10% intervals were similar to quantile regression ($\tau = 0.50$), and those estimated from linear
210 regression (Table 3). The use of the median percentile quantile regression highlights the closeness of linear regression predictions being more indicative of mean values, thus underestimating SOC sequestration potential. The boundary line

estimate of Feng et al. (2013), 0.89 ± 0.05, was nearly double their linear regression; this was not the case in our study. Boundary line analysis uses a subset of data to estimate, in this case, an upper limit, the data set used by Feng et al. (2013) had a wider spread of measured fine fraction OC of 0.9 to 71.7 g C kg^{-1} soil, compared to 1.72 to 18.29 g C kg^{-1} soil in our UK soils. Therefore, the upper subset of data was composed of higher values giving a steeper slope and demonstrates that the range of data biases the C sequestration estimate generated by boundary line analysis.

The strength of using quantile regression analysis is that it makes no assumptions of homogeneity of variance and uses the entire data set to estimate the upper limit of a response. The measured fine fraction OC in the UK sites lacks homogeneity of variance (Fig. 4), where the variation in the measured fine fraction OC increases with the proportion of fine fraction. Standard deviation of the proportion of fine fraction in the 10th percentile is 0.4 compared to 6.9 in the 90th percentile. Of the methods explored in this study for our grassland soils, we consider the quantile regression at the 90th percentile estimate of maximum fine fraction OC to be the most robust. This method results in the greatest number of unsaturated samples (Table 4) suggesting great potential for additional sequestration.

When examining the estimated OC input versus existing fine fraction OC using estimates generated by quantile regression at the 90th percentile, a positive correlation between current fine fraction OC and estimated C input (Kendall tau (τ);0.323, P < 0.001), was observed for the entire data set. This was not the case at the site level (Fig. A1). In some instances, increasing fine fraction OC (g C kg^{-1} soil) was associated with increased estimated C input until saturation, such as Aberyswyth, Myerscough and Plumpton. Despite a higher fine fraction OC content, these samples are furthest from saturation. In contrast the opposite was true for Crichton and Hillsborough (and Harpenden, Kirkton and Overton, although not statistically significant) implying that for these sites, samples with a higher fine fraction OC are closer to, or over saturation. It is unclear why this is the case particularly as in all sites, bar Harpenden, there is a positive regression between mass proportion of the fine fraction and fine fraction OC (Table A3). Meaning that higher fine fraction OC is also associated with higher mass proportion of the fine soil fraction. It is likely that the OM input to the soils with the higher mass proportion of fine fraction is insufficient to bridge the gap between current and estimated maximum fine fraction OC, as it is not possible to identify any other effect due to pedogenic or environmental conditions measured in this work. Further work investigating grasslands with similar soil types and textures, and environmental conditions, but contrasting management in terms of fertiliser regimes, grazing densities, sward composition and management, may help to elucidate management factors that can be used to increase fine fraction OC and explain the differences observed in this work.

Estimating maximum fine fraction OC based on mass proportion of fine fraction is likely to be an oversimplification of the dynamics of fine fraction OC accrual. Other parameters such as mineralogy, soil microbial community, environmental conditions (e.g. precipitation, Table 2) and land management, can significantly influence fine fraction OC stabilisation (Cotrufo et al., 2015; Kallenbach et al., 2016). This work has identified some soil and environmental properties that may play a role in fine fraction OC stabilisation such as median annual temperature, %N and %C in the bulk soil, mean annual rainfall and %N

in the fine fraction (Table 2). Warmer median annual temperatures may enhance plant productivity and microbial processing, the by-products of which are important precursors to fine fraction OC (Cotrufo et al., 2013). It would be interesting to know at which point higher temperatures have a deleterious effect on fine fraction OC accumulation. Mean annual rainfall and %N in the fine fraction were negatively correlated to fine fraction OC. It was anticipated that fine fraction OC would be positively correlated with fine fraction N, as N rich microbial by-products have been found to form new organo-mineral associations onto which OC preferentially binds (Kopittke et al., 2018). These bonds may have been disturbed during the fractionation process, resulting in an N rich fine fraction with less OC content.

The influence of soil type on fine fraction OC was also evident in our results as all soil types had statistically significant positive correlations between the mass proportion of fine fraction and measured fine fraction OC, except for leptosols and luvisols (Fig. 2). However, these soil types exhibited the greatest proportion of total SOC stabilised in the fine fraction (Fig.3). Luvisols have a high base saturation facilitating more fine fraction OC stabilisation via complexation of organic ligands by free Ca^{2+} (Chen et al., 2020). Identifying soils where a greater proportion of total SOC is stored in the fine fraction is important to recognise where fine fraction OC needs to be protected, but also where it can be enhanced.

Whilst we consider the quantile regression at the 90th percentile method to provide the most robust estimate of maximum fine fraction OC in the sites studied, further experimental work to test the saturation level of these soils, would help to validate this. Incubation studies that force an unsaturated soil to its 'saturation' level and the effect of influencing variables, mentioned above will help to elucidate the factors controlling fine fraction OC saturation. Further empirical evidence of practical methods to manipulate fine fraction OC stabilisation processes to promote the formation of new organo-mineral associations, and understanding their stability is necessary to guide grassland management to enhance SOC sequestration.

4.3 Effect of sward age on fine fraction OC

It was anticipated that for fields of an older sward age, a greater proportion of total SOC would be stabilised as fine fraction OC, as tillage breaks up macroaggregates making OC in the fine fraction available for mineralisation. Alternatively, fine fraction OC is less sensitive to disturbance than particulate organic matter (POM), resulting in the accumulation of POM as the fine fraction OC pool remains stable, if sufficiently saturated. The results seem to support neither hypothesis. The proportion of total SOC stabilised in the fine fraction was not consistently higher in the oldest field, and in some instances was significantly less, such as Aberystwyth (Table A2). When grouped in five year intervals, significant differences in C:N ratio of the fine fraction, the proportion of fine fraction in a sample (%) by mass, measured fine fraction OC (g C kg^{-1} soil) and the relative proportion of measured fine fraction OC of the total SOC content of the bulk soil were found between age groups (Table 5). However, there was no consistent trend in the results. This data does not support the hypothesis that older swards will have a greater proportion of SOC stabilised in the fine soil fraction, and a reduced potential for additional C sequestration.

Equally, there was no negative correlation between sward age and the proportion of total SOC stabilised which would be supportive of the alternate hypothesis. From the data, fine fraction OC makes up a greater proportion of SOC with increasing sward age when comparing the less than 5 years, 6 to 10 and 11 to 15 years age groups. However there is a significant decrease in the amount of SOC that is stabilised in the fine fraction in the 16 to 20 years group, this is likely due to fields in this age range originating from Crichton, Hillsborough and Plumpton, which have some of the lowest mass proportion of the fine fraction (Figure 1, C). The sward age analysis may also be confounded by the variation of the proportion of fine fraction, particularly on soil properties influenced by mass proportion of fine fraction such as %C and %N and current fine fraction OC (g C kg^{-1} soil). However, it was not possible to conduct robust ANCOVA's with a grouping variable with more than two levels. It may be possible to elucidate the relationship better from a wider study with more samples per age group as our 16 to 20 years group only has 9 values compared to 48 in the less than 5 years group.

Fine fraction OC only accounted for 4.5 to 50.12% indicating high OC storage in other soil pools such as POM, or different aggregate fractions. The fine roots of grassland flora species promote aggregate formation (O'Brien and Jastrow, 2013; Rasse et al., 2005), which may be a dominant stabilisation process in grasslands. However previous work has found no effect of sward age or the frequency of grassland reseeding on the % C in differing aggregate fractions (> 2000 μm, 250–2000 μm, 53–250 μm and < 53 μm) (Carolan and Fornara, 2016; Fornara et al., 2020). The impact of reseeding disturbance may be offset due to the high density of roots in grasslands by facilitating aggregate reformation. Additionally, dissolved organic carbon from below ground inputs is more efficiently stabilised in organo-mineral associations than above ground dissolved organic carbon (litter leachate) (Sokol and Bradford, 2019). The narrow rhizosphere to bulk soil ratio in grasslands, may make the fine fraction OC in grasslands more resilient to disturbance events.

5. Conclusions

Estimating the long-term sequestration of soil C in the fine fraction is difficult due to the lack of reliable methodologies that can be widely applied to all soils. Our study has demonstrated that the Hassink (1997) linear regression equation is not suitable to estimate maximum fine fraction OC in a range of UK grassland soils. Therefore, caution should be applied to estimates of maximum fine fraction OC obtained using the Hassink (1997) equation, in instances where it may not accurately reflect fine fraction OC of the soil *in situ*. After exploring various univariate estimation methods, we recommend the use of quantile regression at the 90th percentile to overcome the shortfalls of least squares linear regression. However, such a simple estimate is unlikely to accurately reflect the dynamics of fine fraction OC stabilisation. This work has helped to identify some key parameters that play a role in fine fraction OC stabilisation, such median annual temperature, mean annual precipitation, bulk soil %C and %N and fine fraction %N. Further work to understand how these parameters influence fine fraction OC dynamics, will help to accurately assess the feasibility of achieving soil carbon sequestration targets. Our results showed little evidence

305   of the impact of time since last reseeding event on the OC in the fine soil fraction. However, improving our understanding of SOC stabilisation processes, and their resilience to grassland management is essential to ensure that current SOC is not only enhanced but also protected.

Author contribution

KCP, SB, JMC, RMR and EMB formulated the research question and study design. KCP conducted the experimental work,
310   data analysis, and prepared the manuscript draft. All authors contributed to editing and reviewing of the manuscript.

Data Availability

All data resulting from this study are available from the authors upon request to Sarah Buckingham (sarah.buckingham@sruc.ac.uk)

Competing Interest

315   The authors declare that they have no conflict of interest.

Acknowledgements

We acknowledge funding from Business Environment, Industry and Strategy, Ricardo AEA, Scottish Government's Strategic Research Programme and the Global Academy of Agriculture and Food Security, University of Edinburgh. We are grateful to John Parker and Lydia Guo for their assistance in both the field and laboratory. We also thank Mr Steve Freeman for technical
320   assistance, and Margaret Glendining and Sarah Perryman for access to information and data from the Electronic Rothamsted Archive (e-RA). The Rothamsted Long-term Experiments National Capability (LTE-NCG) is supported by the UK Biotechnology and Biological Sciences Research Council (BBS/E/C/000J0300) and the Lawes Agricultural Trust. The authors are also grateful to collaborators at the local field sites for supplying soil samples and management information.

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Table 1. Summary of UK grassland site characteristics.

Site	Age range (years)	Land Use[a]	Mean Annual Temperature (ºC)[b]	Mean Annual Rainfall (mm)[a]	Elevation (m.a.s.l)	WRB Soil Type[c]	Soil Texture[c]
Aberystwyth (52°25'N 04°02'W)	2 to 33	UpG	9.5 to 11	1000	20 to 65	ST, CM	Clay to sandy loam
Crichton (55°02'N 03°35'W)	1 to 20	DP	9.5 to 9.9	1100	5 to 50	CM	Clay loam to sandy loam
Easter Bush (55°51'N 03°52'W)	3 to 6	MG	6 to 9	< 700	215 to 265	GL	Clay loam to sandy loam
Harpenden (51°48'N 00°22'W)	22 to 179	UnG	9.5 to 10.5	700	120 to 130	LV	Silty clay loam
Hillsborough (54°27' N 6°04' W)	1 to 37	DP	8.5 to 10	900	120	CM	Clay loam
Kirkton (56°25'N 04°39'W)	1 to 35	UpG	8 to 9.4	2528	163 to 170	PZ	Clay loam to sandy loam
Llangorse (51°55'N 03°16'W)	2.5 to 25	MG	8 to 10	1000		CM	Loam/ Clay to Silty loam
Myerscough (53°51'N 02°46'W)	2 to 48.4	MG	9 to 10.5	1000	8 to 15	GL	Clay to sandy loam
Overton (51°48'N 02°08'W)	3 to 50	MGO	9 to 11	800	240 to 276	LP	Clay loam to silty loam
Plumpton (50°54'N 00°04'W)	1 to 20	MG	9.5 to 11	800	49 to 85 &160 to 215	ST	Clay to clay loam, Chalky clay to chalky loam

[a] Land Use; DP; Dairy pasture, MG; Mixed grazing; MGO; Mixed grazing organic, UpG; Upland grazing, UnG; Ungrazed.

[b] Mean annual temperature and rainfall estimated from Met Office climatic region summaries, averaged over 1981 to 2010.

[c] World Reference Base (WRB) Soil Type: ST; Stagnosols, CM; Cambisols, GL;Gleysol, LV; Luvisols; PZ; Podzol; LP; Leptosol. Soil type and texture determined from GPS locations and UK Soil Observatory Map viewer.

Table 2. Correlation matrix of Kendal tau (τ) coefficients for bulk and fine fraction (< 20 μm) soil properties, sward age and known environmental parameters.

				Bulk Soil						Fine Fraction		
		Temp.	Prec.	Age	%N	%C	C:N	pH	%SC	Fine fraction OC	%N	%C	C:N
Bulk soil	Temp.	1											
	Prec.	-0.05	1										
	Age	0.15	-0.11	1									
	%N	0.23***	-0.07	0	1								
	%C	0.16*	0.06	0.04	0.73***	1							
	C:N	-0.25***	-0.05	0.02	-ᵃ	-ᵃ	1						
	pH	0.07	-0.30***	-0.07	-0.04	0.03	0.02	1					
	%SC	0.26***	-0.43***	0.14*	0.12	0.12	-0.01	0.21***	1				
	Fine fraction OC	0.13*	-0.36***	0.1	0.26***	0.27***	0.01	0.12	-ᵃ	1			
Fine Fraction	%N	-0.32***	0.28***	-0.07	0.17**	0.14*	-0.02	-0.27***	-0.47***	-0.15**	1		
	%C	-0.33***	0.25***	-0.09	0.18**	0.17**	0.06	-0.25***	-0.47***	-ᵃ	0.87***	1	
	C:N	-0.21***	-0.08	-0.16	0.11**	0.21***	0.30***	-0.05	-0.15*	-0.02	-ᵃ	-ᵃ	1

430 [a] No correlation calculated as one variable used to calculate the other.

Age; years since last reseeding event, Temp; median value from the mean annual temperature range (ºC), Prec.; mean annual rainfall (mm), %SC; mass proportion of fine fraction in a sample (%), Fine fraction OC; measured fine fraction OC (g C kg^{-1} bulk soil).

Level of significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$

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Table 3. Analyses coefficients for the estimation of maximum fine fraction organic carbon by linear regression (LR), linear regression with forced zero intercept (LR_0), boundary line (BL) and quantile regression (QR). Lettering indicates slopes that were significantly different within a method ($P < 0.05$).

Method		Slope (± 1 SEM)	*P* slope	Intercept (± 1 SEM)	*P* intercept	RMSE	n	R²
LR	Hassink, (1997)	0.37[a]		4.09			40	
	All UK	0.32 ± 0.023[b]	***	2.86 ± 0.368	***	2.58	129	0.61
LR_0	Hassink, (1997)[a]	0.45 ± 0.02	***			4.97	40	0.94
	All UK	0.47 ± 0.017	***			3.13	129	0.85
BL	5% intervals	0.48 ± 0.058	***			5.89	19	0.79
	10% intervals	0.48 ± 0.070	***			6.36	15	0.77
	15% intervals	0.56 ± 0.056	***			4.77	14	0.89
QR	QR ($\tau = 0.90$)	0.92 ± 0.071	***			7.90	129	0.90
	QR ($\tau = 0.50$)	0.49 ± 0.032	***			3.15	129	0.66

445 RMSE, root mean square error.

Level of significance: *** $P < 0.001$

[a] Data extracted from Hassink, (1997) used to generate slope value with forced zero intercept.

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Table 4. Carbon saturation ratios calculated from the estimated maximum fine fraction organic carbon by linear regression (LR), linear regression with forced zero intercept (LR_0), boundary line (BL) and quantile regression (QR). Values < 1 indicate unsaturated, = 1 at saturation and > 1 are oversaturated samples.

Method		No. of unsaturated samples (n = 129)	Mean ratio	Median
LR	Hassink, (1997)	105	0.77	0.73
	UK	75	0.98	0.94
	UK site specific	71	1	0.99
Forced 0 intercept				
LR_0	Hassink (1997)	30	1.52	1.44
	UK	34	1.47	1.39
	UK site specific	57	1.09	1.04
BL	5%	38	1.42	1.34
	10%	36	1.43	1.35
	15%	50	1.22	1.15
QR	50th	38	1.4	1.32
	90th	99	0.74	0.7

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470 **Table 5. Effect of sward age grouped at five year intervals on selected soil properties. Values are means ± standard error of the mean, and different letters indicate age groups which are significantly different ($P < 0.05$), by columns.**

Age	n	C:N	% SC	Fine fraction organic carbon (g C kg^{-1} soil)	Fine fraction organic carbon (% of SOC$_{total}$)
0 to 5	48	10.18 ± 0.15^a	10.00 ± 1.41^a	5.68 ± 0.49^a	18.32 ± 1.5^{ab}
6 to 10	18	9.79 ± 0.26^{ab}	14.47 ± 1.69^b	8.58 ± 0.59^b	24.94 ± 1.35^c
11 to 15	15	9.33 ± 0.11^b	15.27 ± 2.98^{ab}	9.17 ± 1.66^{ab}	20.66 ± 2.55^{abc}
16 to 20	9	10.41 ± 0.31^a	6.10 ± 0.77^a	4.68 ± 0.44^a	11.54 ± 1.12^a
21+	39	9.50 ± 0.11^b	15.19 ± 1.69^b	7.44 ± 0.69^{ab}	23.21 ± 1.94^{bc}

Age; years since last reseeding event, C:N ratio of the fine fraction, %SC; proportion of fine fraction in a sample (%) by mass, Fine fraction organic carbon (% of SOC$_{total}$); relative proportion of measured fine fraction OC of the total SOC content of the bulk soil.

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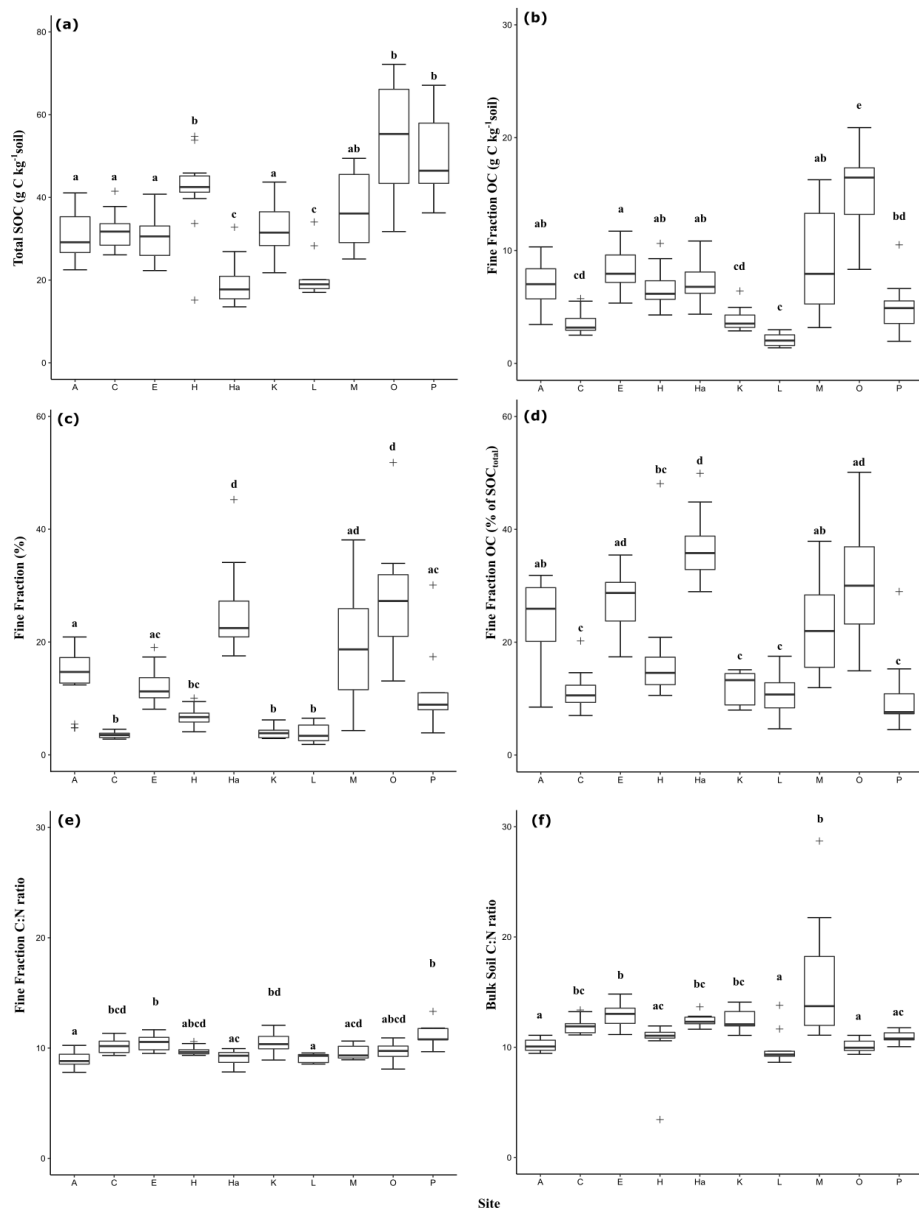
Figure 1. Measured total SOC (g C kg^{-1}soil) (a), total fine fraction organic carbon (g C kg^{-1}soil) (b), mass proportion of fine fraction (< 20 μm, %) (c), relative proportion of measured fine fraction organic carbon of the total SOC content of the bulk soil (d), fine fraction C:N ratio (e) and bulk soil C:N ratio (f), for each of the grassland sites; Aberyswyth (A), Crichton (C), Easter Bush (E), Hillsborough (H), Harpenden (Ha), Kirkton (K), Llangorse (L), Myerscough (M), Overton (O) and Plumpton (P). Boxes represent the 25th and 75th percentile, with lines showing the median value. Whiskers show the lowest and highest values with outliers indicates as crosses (> 1.5 times the interquartile range). Lettering indicates significant differences between soils (P < 0.05).

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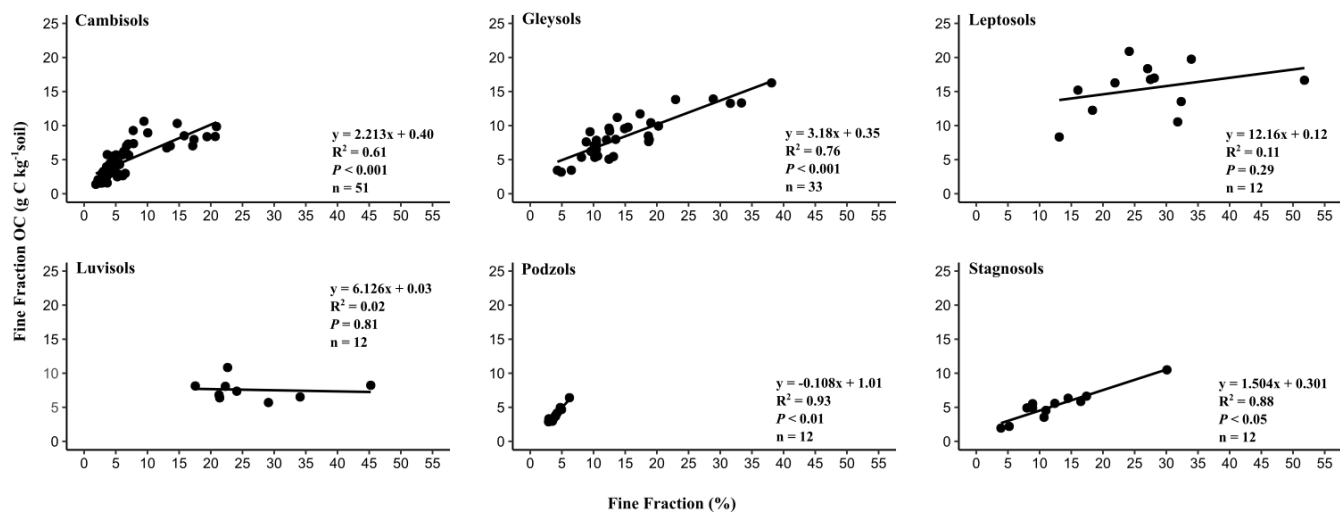
Figure 2. Relationships between mass proportion of the fine fraction (%) and fine fraction organic carbon (g C kg^{-1} soil) in the soil types used in this study.

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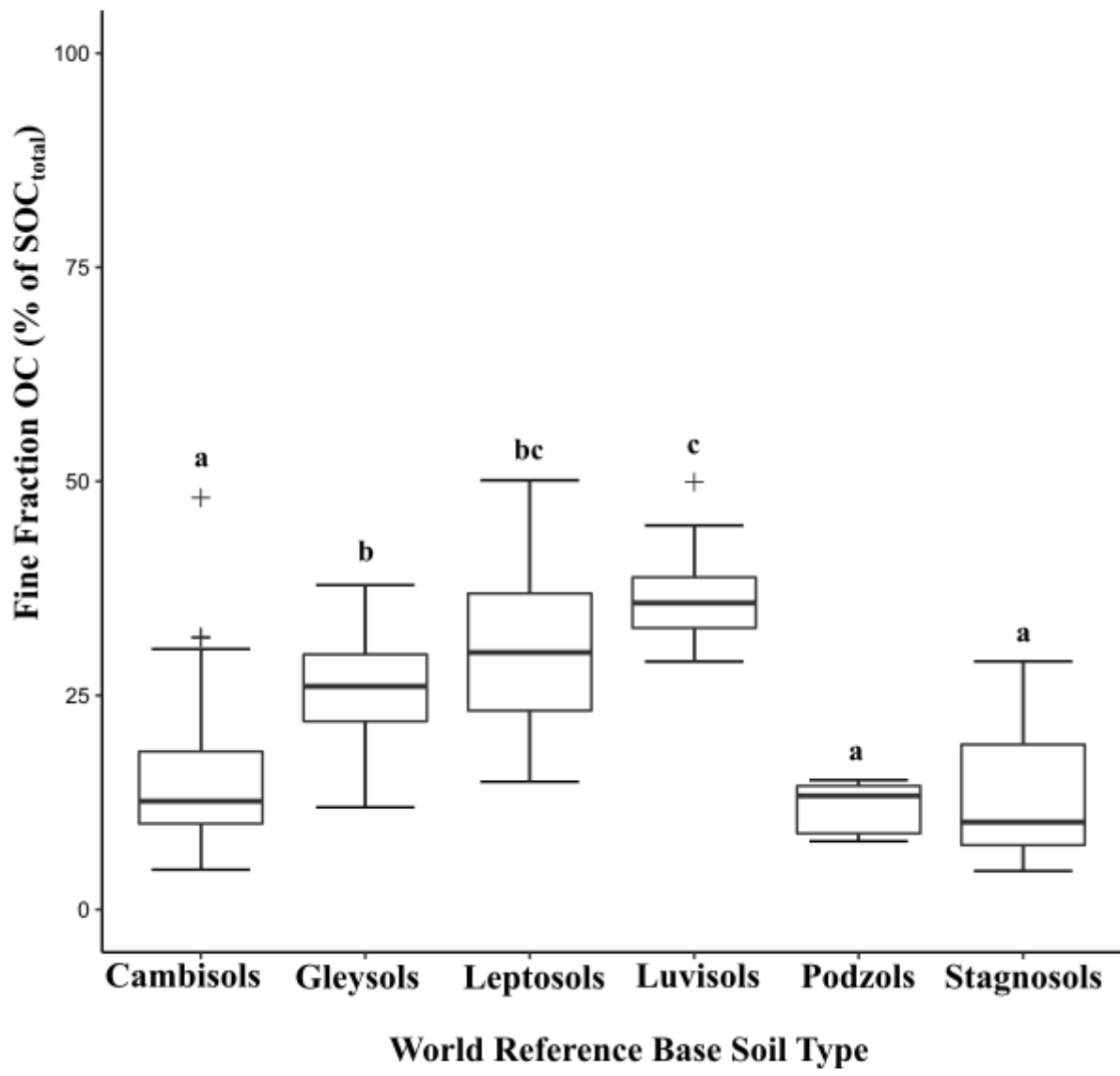
Figure 3. Relative proportion of measured fine fraction organic carbon of the total SOC content of the bulk soil for the different soil types used in this study. Lettering indicates significant differences at $P < 0.05$.

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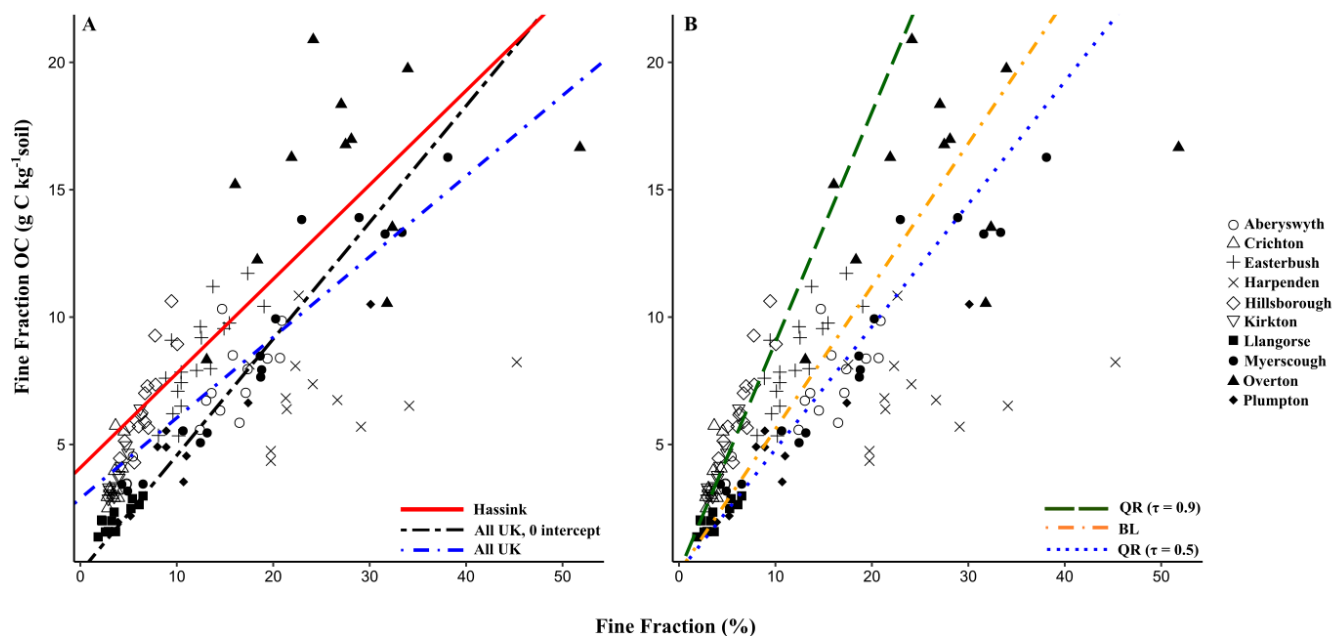
Figure 4. Measured fine fraction organic carbon (g C kg⁻¹ soil) in relation to mass proportion of fine fraction of a soil sample (%). Line of best fit represent (A) linear regression method of Hassink, (1997) and data from this study, and (B) boundary line (BL) using 15% intervals, and quantile regression analysis (QR) at 90ᵗʰ and 50ᵗʰ percentiles.

Appendix A

Table A1. Bulk soil properties for each UK site. Values are means of ten replicates in each field, ± one standard error of the mean. Except Harpenden where values are means of five replicates per field. Lettering indicates values that are significantly different, within a site ($P < 0.05$).

Site	Age (years)	BD[a]	pH	C:N	C (g C kg^{-1} soil)	C stock (t C ha^{-1})	N stock (t N ha^{-1})
Aberyswyth	2	1 ± 0.01^a	5.20 ± 0.05^a	9.70 ± 0.05^b	26.95 ± 0.63^b	73.61 ± 1.73^b	7.59 ± 0.16^b
	6	0.98 ± 0.04^a	4.70 ± 0.04^{bc}	9.68 ± 0.08^b	26.7 ± 0.82^b	73.86 ± 2.28^b	7.62 ± 0.18^b
	11	0.82 ± 0.05^b	5.12 ± 0.06^a	10.46 ± 0.09^a	29.72 ± 0.83^b	76.97 ± 2.15^b	7.36 ± 0.20^b
	31	0.74 ± 0.05^b	4.99 ± 0.09^{ab}	10.54 ± 0.22^a	29.4 ± 1.63^b	74.23 ± 4.12^b	7.01 ± 0.31^b
	33	0.69 ± 0.03^b	4.18 ± 0.02^c	10.59 ± 0.10^a	38.19 ± 1.97^b	95.67 ± 4.92^a	9.01 ± 0.40^a
Crichton	1	0.92 ± 0.03	5.14 ± 0.03^{ab}	12.19 ± 0.08^{ab}	34.66 ± 0.66^a	82.40 ± 1.56	6.76 ± 0.11^b
	3	0.99 ± 0.07	5.65 ± 0.06^b	11.73 ± 0.11^{bc}	29.94 ± 1.37^{ab}	74.63 ± 3.41	6.36 ± 0.26^b
	15	0.93 ± 0.05	4.77 ± 0.04^{ac}	9.90 ± 0.88^c	30.85 ± 3.06^b	79.73 ± 7.91	7.98 ± 0.23^a
	20	0.93 ± 0.04	4.54 ± 0.03^c	13.21 ± 0.14^a	27.26 ± 0.87^b	66.62 ± 2.11	5.04 ± 0.15^c
Easter Bush	3	1.02 ± 0.04^{abc}	5.45 ± 0.06^{ab}	13.03 ± 0.11^{bc}	32.46 ± 1.29^a	93.52 ± 3.72^a	7.17 ± 0.26^a
	5	1.19 ± 0.03^a	5.44 ± 0.06^{ab}	12.84 ± 0.21^{bc}	26.41 ± 0.54^b	74.45 ± 1.52^b	5.80 ± 0.10^{bc}
	5	0.84 ± 0.06^c	5.67 ± 0.04^a	11.74 ± 0.17^d	27.50 ± 1.0^b	58.15 ± 2.12^c	4.94 ± 0.14^d
	6	0.96 ± 0.05^{bc}	5.32 ± 0.06^b	12.45 ± 0.13^c	30.46 ± 1.93^{ab}	71.16 ± 4.50^{bc}	5.72 ± 0.36^{cd}
	6	1.12 ± 0.05^{ab}	5.81 ± 0.20^a	14.15 ± 0.13^a	28.95 ± 0.99^{ab}	75.69 ± 2.59^b	5.35 ± 0.17^{cd}
	8	1.12 ± 0.03^{ab}	4.99 ± 0.04^c	13.43 ± 0.11^b	33.03 ± 0.50^a	89.43 ± 1.34^a	6.66 ± 0.10^{ab}
Harpenden	22	1.37 ± 0.07	7.37 ± 0.04^a	12.09 ± 0.2	16.06 ± 0.59^c	25.37 ± 0.93^c	3.3 ± 0.12^c
	68	1.12 ± 0.08	5.85 ± 0.12^{ab}	12.34 ± 0.08	19.8 ± 0.63^b	50.49 ± 1.59^b	4.06 ± 0.13^b
	179	1.09 ± 0.14	5.63 ± 0.06^b	12.8 ± 0.26	28.7 ± 1.47^a	72.98 ± 3.74^a	5.89 ± 0.30^a
Hillsborough	1	1.79 ± 0.10	6.31 ± 0.07^a	11.25 ± 0.12^{ab}	46.68 ± 2.04	120.16 ± 5.26^{ab}	10.69 ± 0.51^{ab}
	7	1.88 ± 0.08	5.10 ± 0.04^b	11.46 ± 0.11^b	42.85 ± 1.52	108.86 ± 3.87^b	9.51 ± 0.34^{bc}
	16	1.79 ± 0.05	5.33 ± 0.08^b	10.87 ± 0.06^c	42.36 ± 1.98	111.63 ± 5.21^{ab}	10.27 ± 0.47^{ab}
	23	1.75 ± 0.05	4.76 ± 0.03^c	11.33 ± 0.09^{ab}	46.44 ± 1.78	125.43 ± 4.82^a	11.08 ± 0.45^a
	37	1.69 ± 0.06	5.13 ± 0.06^b	10.34 ± 0.77^{ac}	40.90 ± 3.10	86.04 ± 6.52^c	8.38 ± 0.24^c
Kirkton	1	0.9 ± 0.04	4.78 ± 0.04^c	12.13 ± 0.11^c	27.90 ± 0.81^c	82.03 ± 2.39^b	6.77 ± 0.22
	3	0.95 ± 0.04	5.49 ± 0.06^a	12.61 ± 0.15^b	36.67 ± 1.56^a	98.19 ± 4.17^{ab}	7.79 ± 0.31

	5	0.83 ± 0.06	5.15 ± 0.03[b]	13.56 ± 0.08[a]	34.83 ± 1.84[ab]	103.03 ± 5.45[a]	7.59 ± 0.38
	35	0.97 ± 0.06	4.72 ± 0.07[c]	11.67 ± 0.13[d]	30.51 ± 1.48[bc]	90.50 ± 4.38[ab]	7.72 ± 0.32
Llangorse	2.5	1.01 ± 0.04	5.14 ± 0.08[c]	9.21 ± 0.09	17.83 ± 0.42	49.75 ± 1.18	5.40 ± 0.11[ab]
	5	0.93 ± 0.04	5.44 ± 0.03[b]	9.40 ± 0.07	18.60 ± 0.45	50.80 ± 1.22	5.40 ± 0.10[b]
	15	0.94 ± 0.06	5.68 ± 0.03[a]	9.36 ± 0.17	19.42 ± 0.38	53.70 ± 1.06	5.74 ± 0.12[ab]
	25	1.06 ± 0.03	5.54 ±0.07[ab]	9.16 ± 0.87	19.73 ± 2.52	55.10 ± 7.05	6.18 ± 0.34[a]
Myerscough	2	1.22 ± 0.02[ab]	4.97 ± 0.05[b]	13.58 ± 0.24[bc]	27.47 ± 0.65[c]	82.25 ± 1.96[c]	6.07 ± 0.15[bc]
	6	1.10 ± 0.04[b]	5.59 ± 0.05[a]	11.79 ± 0.76[c]	41.44 ± 2.73[a]	124.05 ± 8.17[a]	10.56 ± 0.28[a]
	13	0.93 ± 0.05[b]	5.00 ± 0.20[b]	13.12 ± 0.43[c]	44.82 ± 2.34[a]	134.45 ± 7.01[a]	10.30 ± 0.71[a]
	34	1.29 ± 0.02[a]	5.99 ± 0.13a	17.20 ± 1.12[ab]	37.58 ± 1.45[ab]	112.46 ± 4.36[ab]	6.71 ± 0.30[b]
	48.4	1.44 ± 0.06[a]	5.77 ± 0.02[a]	22.10 ± 1.46[a]	29.86 ± 1.96[bc]	88.97 ± 5.85[bc]	4.03 ± 0.08[c]
Overton	3	0.98 ± 0.09[a]	6.58 ± 0.12[b]	9.76 ± 0.05[b]	32.77 ± 0.84[c]	83.02 ± 2.13[b]	8.51 ± 0.23[b]
	12	0.38 ± 0.03[b]	6.83 ± 0.03[b]	10.18 ± 0.12[ab]	70.18 ± 1.92[a]	81.20 ± 2.23[b]	7.99 ± 0.23[b]
	22	0.71 ± 0.07[ab]	7.36 ± 0.04[a]	10.68 ± 0.39[a]	59.88 ± 3.86[b]	132.75 ± 8.56[a]	12.33 ± 0.39[a]
	50	1.74 ± 0.9[a]	4.63 ± 0.08[c]	10.14 ± 0.14[ab]	51.18 ± 2.84[b]	153.08 ± 8.50[a]	15.08 ± 0.80[a]
Plumpton	1	0.99 ± 0.02[a]	6.34 ± 0.08[b]	10.85 ± 0.08ab	40.92 ± 1.21[b]	122.21 ± 3.61[b]	11.26 ± 0.28[b]
	5	1.08 ± 0.03a	7.15 ± 0.06a	11.27 ± 0.41a	45.55 ± 0.61[b]	132.09 ± 1.77[b]	11.87 ± 0.48[b]
	20	0.72 ± 0.04b	5.38 ± 0.21c	10.54 ± 0.17b	58.08 ± 2.36[a]	163.23 ± 6.62[a]	15.47 ± 0.56[a]

[a]Bulk density (BD), means and SEM of six samples, except Harpenden with two samples per field, corrected for stones.

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Table A2. Fine fraction (< 20 µm) soil properties for each UK site. Values are means of three replicates in each field, ± one standard error of the mean. Lettering indicates values that are significantly different, within a site ($P < 0.05$).

Location	Age (years)	%N	%C	C:N	% Fine Fraction	Organic Carbon (g C kg^{-1} bulk soil)[a]	Organic Carbon (% of SOC_{total})
Aberyswyth	2	0.48 ± 0.01[b]	4.16 ± 0.08	8.62 ± 0.08[ab]	19.08 ± 1.04[a]	7.93 ± 0.45[a]	0.30 = 0.01[a]
	6	0.52 ± 0.04[b]	4.14 ± 0.30	8.05 ± 0.17[b]	14.47 ± 1.18[ab]	5.92 ± 0.22[ab]	0.21 ± 0.02[ab]
	11	0.55 ± 0.03[b]	4.89 ± 0.25	8.86 ± 0.06[ab]	18.02 ± 1.51[ab]	8.77 ± 0.56[a]	0.29 ± 0.02[ab]
	31	0.61 ± 0.05[ab]	5.78 ± 0.62	9.51 ± 0.30[ab]	13.78 ± 0.49[b]	8.02 ± 1.15[a]	0.27 ± 0.02[ab]
	33	0.76 ± 0.03[a]	7.57 ± 0.37	9.96 ± 0.23[a]	5.02 ± 0.22[c]	3.81 ± 0.36[b]	0.10 ± 0.01[b]
Crichton	1	1.01 ± 0.06	10.53 ± 0.83	10.40 ± 0.23[ab]	4.00 ± 0.45	4.24 ± 0.69	0.12 ± 0.02
	3	1.15 ± 0.27	11.17 ± 2.28	9.84 ± 0.35[b]	3.28 ± 0.23	3.75 ± 1.00	0.13 ± 0.04
	15	1.02 ± 0.12	9.76 ± 1.20	9.52 ± 0.12[b]	3.52 ± 0.26	3.38 ± 0.31	0.10 ± 0.02
	20	0.82 ± 0.05	9.07 ± 0.72	11.03 ± 0.24[a]	3.37 ± 0.3	3.01 ± 0.09	0.11 ± 0.01
Easter Bush	3	0.65 ± 0.04	7.15 ± 0.50	11.00 ± 0.13[ab]	14.38 ± 1.56[ab]	10.27 ± 1.19[a]	0.30 ± 0.01
	5	0.65 ± 0.04	6.91 ± 0.50	10.57 ± 0.06[bc]	12.17 ± 0.9[ab]	8.34 ± 0.43[ab]	0.32 ± 0.02
	5	0.67 ± 0.02	6.62 ± 0.23	9.83 ± 0.07[c]	9.55 ± 0.73[b]	6.32 ± 0.51[b]	0.23 ± 0.03
	6	0.68 ± 0.03	7.81 ± 0.43	9.85 ± 0.24[c]	9.75 ± 0.23[b]	6.88 ± 1.13[ab]	0.23 ± 0.01
	6	0.72 ± 0.12	7.11 ± 1.31	11.43 ± 0.16[a]	10.58 ± 1.04[b]	8.22 ± 0.70[ab]	0.27 ± 0.02
	8	0.59 ± 0.04	6.07 ± 0.30	10.35 ± 0.28[bc]	16.47 ± 1.3[a]	9.91 ± 0.26[ab]	0.30 ± 0.01
Harpenden	22	0.23 ± 0.01[b]	1.90 ± 0.04[c]	8.20 ± 0.26[b]	36.15 ± 4.77[a]	6.82 ± 0.75[ab]	0.42 ± 0.04
	68	0.32 ± 0.01[b]	3.08 ± 0.06[b]	9.54 ± 0.21[a]	22.27 ± 0.92[b]	6.86 ± 0.28[ab]	0.36 ± 0.02
	179	0.46 ± 0.03[a]	4.35 ± 0.36[a]	9.54 ± 0.12[a]	20.83 ± 1.64[b]	9.02 ± 0.91[a]	0.32 ± 0.01
Hillsborough	1	0.90 ± 0.08	8.97 ± 1.14	9.91 ± 0.34	7.37 ± 0.12	6.86 ± 1.93	0.14 ± 0.03
	7	1.04 ± 0.06	10.23 ± 0.91	9.80 ± 0.31	8.05 ± 0.08	8.15 ± 0.96	0.17 ± 0.02
	16	0.99 ± 0.04	9.36 ± 0.32	9.46 ±0.03	6.33 ± 0.19	5.92 ± 0.13	0.15 ± 0.01
	23	1.15 ± 0.01	11.11 ± 0.13	9.70 ± 0.18	4.58 ± 0.27	5.10 ± 0.36	0.12 ± 0.01
	37	1.04 ± 0.04	10.12 ± 0.35	9.76 ± 0.04	7.15 ± 0.33	7.22 ± 0.10	0.27 ± 0.11
Kirkton	1	0.91 ± 0.03	9.27 ± 0.12[b]	10.15 ± 0.24[b]	3.90 ± 0.1	3.62 ± 0.13	0.14 ± 0.01
	3	1.01 ± 0.04	10.63 ± 0.33[a]	10.56 ± 0.27[ab]	3.02 ± 0.03	3.20 ± 0.07	0.08 ± 0.00
	5	0.88 ± 0.03	10.23 ± 0.16[ab]	11.66 ± 0.31[a]	4.62 ± 0.95	4.75 ± 1.03	0.13 ± 0.02

	35	0.96 ± 0.03	9.22 ± 0.40^b	9.64 ± 0.39^b	4.23 ± 0.42	3.93 ± 0.51	0.14 ± 0.00
Llangorse	2.5	0.51 ± 0.03^b	4.76 ± 0.29^b	9.36 ± 0.07	6.00 ± 0.32^a	2.83 ± 0.10^a	0.16 ± 0.01^a
	5	0.88 ± 0.08^a	8.29 ± 0.80^a	9.43 ± 0.07	2.65 ± 0.43^b	2.13 ± 0.11^{ab}	0.11 ± 0.01^{ab}
	15	0.67 ± 0.10^{ab}	6.06 ± 0.78^{ab}	9.11 ± 0.24	3.23 ± 1.03^b	1.81 ± 0.34^b	0.09 ± 0.02^b
	25	0.62 ± 0.06^{ab}	5.32 ± 0.54^b	8.60 ± 0.05	3.27 ± 0.22^b	1.72 ± 0.14^b	0.07 ± 0.01^b
Myerscough	2	0.63 ± 0.08	6.60 ± 0.76	10.43 ± 0.12	5.23 ± 0.66^c	3.35 ± 0.09^c	0.12 ± 0.00^b
	6	0.49 ± 0.03	4.57 ± 0.29	9.31 ± 0.23	27.50 ± 3.85^a	12.39 ± 1.24^a	0.31 ± 0.04^a
	13	0.50 ± 0.05	4.83 ± 0.60	9.52 ± 0.32	30.88 ± 4.39^a	14.45 ± 0.92^a	0.30 ± 0.02^a
	34	0.47 ± 0.01	4.28 ± 0.13	9.12 ± 0.07	18.72 ± 0.04^{ab}	8.02 ± 0.24^b	0.21 ± 0.02^{ab}
	48.4	0.47 ± 0.02	4.47 ± 0.36	9.58 ± 0.36	12.08 ± 0.74^{bc}	5.35 ± 0.14^{bc}	0.17 ± 0.02^b
Overton	3	0.42 ± 0.03^c	3.57 ± 0.31^c	8.45 ± 0.18^b	38.65 ± 6.58	13.57 ± 1.77	0.41 ± 0.05
	12	0.88 ± 0.05^a	8.52 ± 0.59^a	9.64 ± 0.11^a	20.70 ± 2.41	17.45 ± 1.75	0.25 ± 0.02
	22	0.61 ± 0.02^b	6.36 ± 0.18^b	10.36 ± 0.15^a	19.85 ± 4.39	12.52 ± 2.50	0.23 ± 0.07
	50	0.63 ± 0.05^b	6.23 ± 0.29^b	10.04 ± 0.45^a	29.50 ± 2.23	18.29 ± 0.86	0.34 ± 0.04
Plumpton	1	0.35 ± 0.02^b	3.81 ± 0.18^b	10.87 ± 0.09	19.50 ± 5.61	7.23 ± 1.74	0.18 ± 0.05
	5	0.36 ± 0.04^b	4.19 ± 0.49^b	11.76 ± 0.91	6.60 ± 2.08	2.56 ± 0.49	0.06 ± 0.01
	20	0.56 ± 0.02^a	5.96 ± 0.23^a	10.75 ± 0.62	8.60 ± 0.3	5.11 ± 0.21	0.08 ± 0.01

% Fine Fraction; mass proportion of fine fraction in a sample (%).

[a] MAOC (g C kg^{-1} bulk soil) accounts for the proportion of fine fraction per kilogram of bulk soil.

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Table A3. Linear regression coefficients for the estimation of maximum fine fraction organic carbon (g C kg $^{-1}$ soil). Lettering indicates slopes that are significantly different ($P < 0.05$).

Site	Slope (± 1 SEM)	*P* slope	Intercept (± 1 SEM)	*P* intercept	RMSE	n	R^2
Aberyswyth	0.33 ± 0.059[bc]	***	2.28 ± 0.892	*	1.11	15	0.70
Crichton	1.14 ± 0.470[abcd]	*	-0.44 ± 1.684	Ns	0.79	12	0.37
Easter Bush	0.49 ± 0.094[d]	***	2.33 ± 1.172	Ns	1.10	18	0.63
Harpenden	-0.02 ± 0.07[a]	Ns	8.01 ± 1.837	**	1.42	9	0.01
Hillsborough	0.97 ± 0.148[d]	***	0.16 ± 1.02	Ns	0.84	15	0.77
Kirkton	1.01 ± 0.088[abcd]	***	-0.11 ± 0.357	Ns	0.26	12	0.93
Llangorse	0.29 ± 0.055[abc]	***	1.03 ± 0.225	***	0.27	12	0.73
Myerscough	0.40 ± 0.031[bcd]	***	1.07 ± 0.669	Ns	1.14	15	0.93
Overton	0.12 ± 0.109[cd]	Ns	12.16 ± 3.142	**	3.35	12	0.11
Plumpton	0.30 ± 0.042[ab]	***	1.45± 0.573	*	0.82	9	0.88

555   RMSE: Root mean square error.

Level of significance: * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$

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Table A4. Linear regression coefficients for the estimation of maximum fine fraction organic carbon (g C kg $^{-1}$ soil) with a forced zero intercept. Lettering indicates slopes that are significantly different ($P < 0.05$).

Site	Slope (± 1 SEM)	P	RMSE	n	R^2
Aberyswyth	0.47 ± 0.024[bc]	***	1.357	15	0.96
Crichton	1.02 ± 0.067[cdef]	***	0.796	12	0.95
Easter Bush	0.67 ± 0.024[e]	***	1.231	18	0.98
Harpenden	0.26 ± 0.035[a]	***	2.739	9	0.87
Hillsborough	0.99 ± 0.033[f]	***	0.842	15	0.99
Kirkton	0.98 ± 0.0197[def]	***	0.265	12	0.99
Llangorse	0.52 ± 0.035[abcdef]	***	0.474	12	0.95
Myerscough	0.45 ± 0.016[b]	***	1.255	15	0.98
Overton	0.52 ± 0.055[bcd]	***	5.297	12	0.89
Plumpton	0.39 ± 0.030[ab]	***	1.141	9	0.96

RMSE: Root mean square error.

Level of significance: * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$
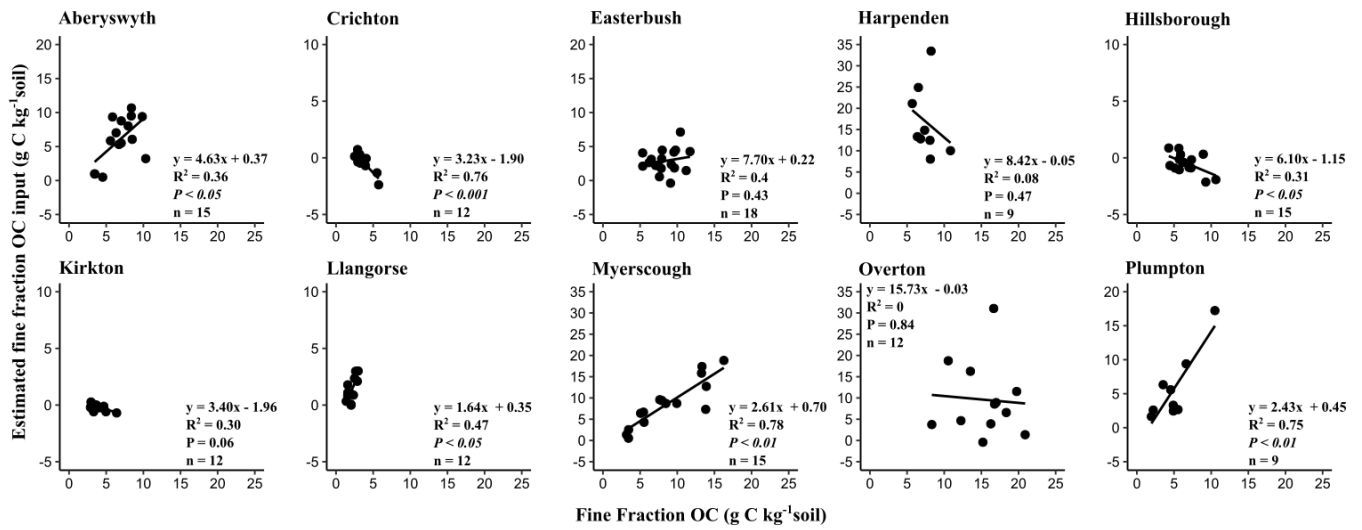
Figure A1. Estimated fine fraction OC input (g C kg⁻¹ soil) compared to measured fine fraction OC (g C kg⁻¹ soil) in each of the sites studied. The estimated fine fraction OC input (g C kg⁻¹ soil) was calculated by subtracting the maximum fine fraction OC (g C kg⁻¹ soil) from the current fine fraction OC (g C kg⁻¹ soil). The maximum fine fraction OC (g C kg⁻¹ soil) was estimated using the quantile regression equation (τ = 0.90), where, maximum fine fraction OC = 0.92 multiplied by the mass proportion fine fraction (%).