

Response to Reviewer 1 Comments on Manuscript, bg-2020-273

Estimating maximum mineral associated organic carbon in UK grasslands

Kirsty C. Paterson, Joanna M. Cloy, Robert. M. Rees, Elizabeth M. Baggs, Hugh Martineau, Dario
5 Fornara, Andrew J. Macdonald, and Sarah Buckingham

We thank the reviewers for their comments and evaluation of our manuscript. Please find below our response to
comments made by reviewer 1. Reviewer's comments are in black text, and our responses are in blue text, changes
10 to the manuscript are highlighted in yellow. Line numbers refer to the revised manuscript (marked version) below.

Overall Comments:

The manuscript under review investigates the mineral associated organic carbon (MAOC) distribution in grassland
soils of varying sward age, across the UK. The authors compared the Hassink's reference equation to calculate the
15 saturation capacity against alternative methods, which showed a more accurate assessments of carbon sequestration
potential. The paper is of good quality, with a robust methodology and well written and developed in each sections.
I do not have any major concerns but, rather, some points of discussion as following:

The forced intercept to 0 is generally suggested to avoid the paradox of having MAOM without any fine (silt and
20 clay) fractions. However, in my experience with very large datasets, I have never seen a soil without any fine
fraction (at least temperate soils covered with any type of vegetation). It seems that the saturation equation is a type
of function where the x domain is always >0 . Indeed, the authors forced the intercept to 0 using the BL and QR
methods, therefore, it would be worth to have a more in depth elaboration of this choice.

We agree with this comment, which was outlined in the introduction, lines 73 to 74: "Using a forced zero intercept
25 overcomes the contradiction of a positive intercept indicating the presence of MAOC without any fine soil fraction
(Beare et al., 2014; Feng et al., 2013; Liang et al., 2009)".

The following has been added to section 2.3.1, line 168.

“Forcing the intercept to zero overcomes the paradox of having C stabilised as MAOC without any fine fraction in

30 the soil.”

In the paragraph in line 255, the authors reported: “The C:N ratio of MAOC was 9.84 ± 1.00 (mean \pm standard deviation) falling within the typical C:N range of fungi (4.5 to 15) whilst bacteria have a lower C:N ratio of 3 to 5 (Cotrufo et al., 2019), suggesting that the MAOC in the grasslands is predominantly of fungal origin.” Indeed, this
35 is an erroneously interpretation as MAOM is not entirely composed of living microbial biomass. C:N around 9 is on the average of European grassland (Figure 3 of Cotrufo et al., 2019), while other systems ‘fungal-dominated’ such a coniferous forests have a much higher C:N ratio. By the way, it would be interesting to know if C:N of MAOM differs significant across sites.

The C:N of MAOM does differ significantly between the selected sites. An extra figure, E has been added to the
40 panel in figure 1, see below. The following has been added to the results section 3.1, lines 190 to 193.

“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 C:N ratio was significantly different between the sites, Figure 1, however the mean of all the data showed little deviation,
45 9.84 ± 1.00 (mean \pm standard deviation).”

As suggested by both reviewers the comment regarding the C:N ratio and potential origin of the OC has been removed.

50

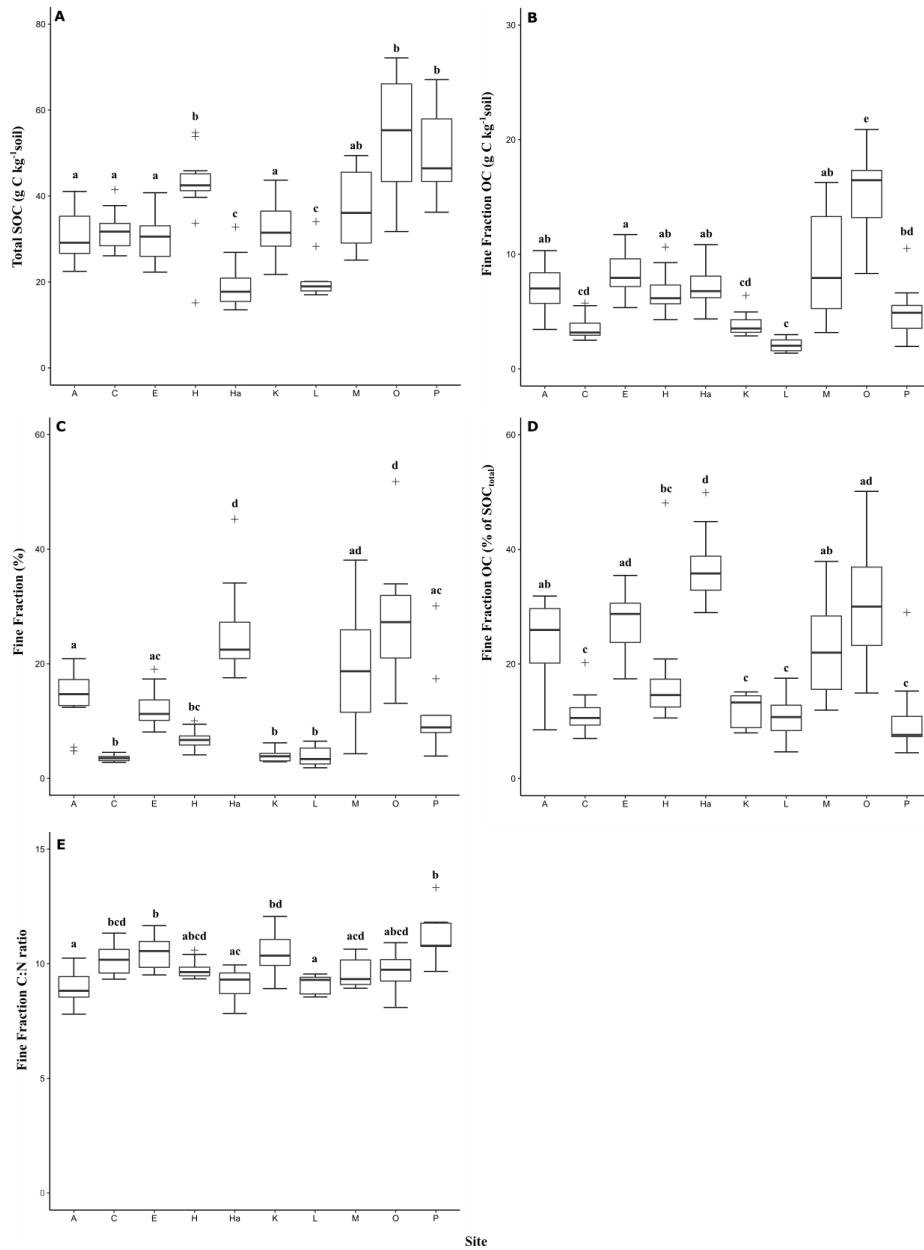


Figure 1. Measured total SOC (g C kg⁻¹soil) (A) total fine fraction organic carbon (g C kg⁻¹soil) (B), mass proportion of fine fraction (< 20 μ m, %) (C), relative proportion of measured fine fraction organic carbon of the total SOC content of the bulk soil (D) and fine fraction C:N ratio (E), for each 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 indicated as crosses (> 1.5 times the interquartile range). Lettering indicates significant differences between soils ($P < 0.05$).

60

The difference between the Hassink's and UK equation implicitly suggests that a universal saturation equation likely does not exist, but many equations are controlled by interacting factors as mineralogy, soil microbial community etc. This concept is developed around line 215 but the conclusion of the paragraph is quite elusive. I would encourage the authors to develop 'a way forward paragraph' that can guide a future research. I imagine, 65 for instance, incubation experiments with unsaturated soils (according to those equations) where excess of high quality inputs are applied to see their 'real' saturation level. In this context, I wonder if authors can produce a plot of MAOM vs estimated C input, which may reveal (or not) some interesting correlations.

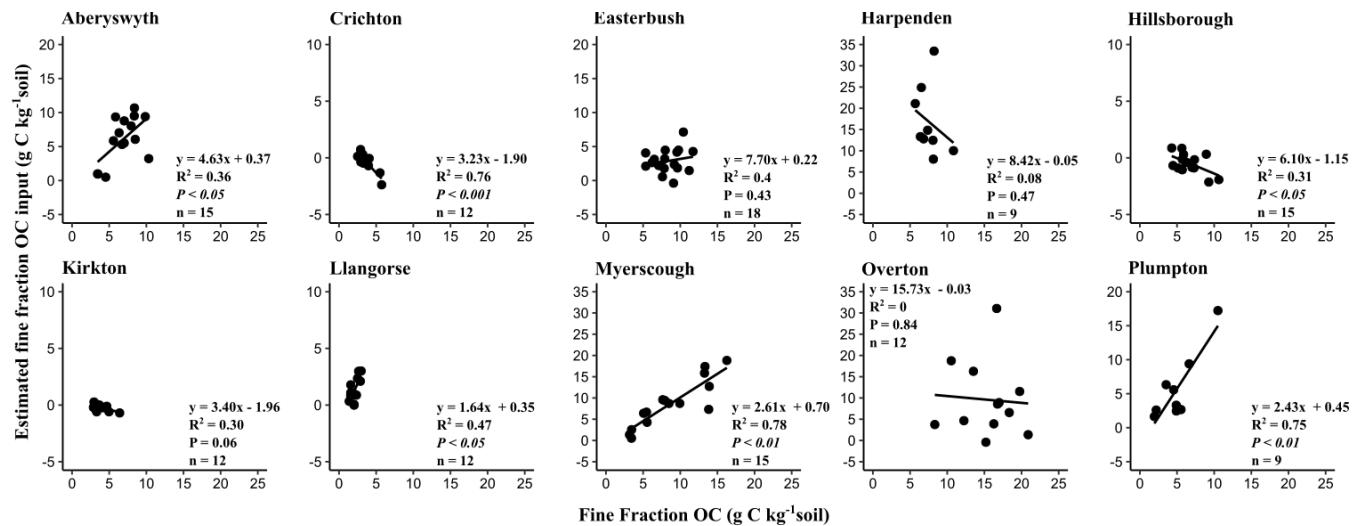
The following has been added to the discussion, lines 301 to 307.

70 "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 which 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. In particular further empirical evidence of how to manipulate fine fraction OC stabilisation processes in a way that 75 is practical for grassland management to promote the formation of new organo-mineral associations, and understanding their stability will be important for establishing the true potential of additional carbon sequestration across managed grasslands."

80 A plot as advised has been produced, however it was felt to be more beneficial to present it on a site basis. The y axis is not consistent in each graph as it was impossible to tell the data points apart in some instances using one scale for all. See lines 270 to 282 in the revised manuscript.

85 "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. However, this was not the case at the site

level, see Fig. A1. Where in some instances increasing fine fraction OC (g C kg^{-1} soil) was associated with increased estimated C input until saturation, such as Aberystwyth, Myerscough and Plumpton. Therefore, despite a higher fine fraction OC contents 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 90 these sites samples with a higher fine fraction OC are closer to 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 95 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.”



100 **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 OC input was predicted using quantile regression at the 90th percentile.**

105 In the conclusion, the QR method is recommended but is not indicated at which quantile level. This makes a substantial difference in the relative proportion of saturated soils (table 4) and, hence, a possible perception of policy priorities. Are the soils mostly saturated or not? Is the index robust enough to provide management guidance? As is it, the conclusion left me a little bit hanging.

The QR percentile has been specified throughout the discussion where use of QR is recommended.

110

Lines 268 and 269 have been amended and incorporated into the following paragraph, see changes highlighted in yellow below, to make it clear that of the methods explored in this work, the QR at the 90th percentile is the most robust. But all of the methods over simplify the dynamics of MAOC accrual, therefore a greater understanding of how, and if, MAOC stabilisation processes occur through further research will make it possible to consider if this
115 is a priority for carbon sequestration policies.

“Therefore, 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.”

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This position is then summarised in the conclusion, lines 350 to 356.

“After exploring various univariate estimation methods we recommend the use of quantile regression at the 90th percentile to overcome the shortfalls of 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 which
125 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”.

Specific Comments:

130 Line 78, hypothesis ii: I see also the way around. Since MAOC is less sensitive to disturbance (than POM), the ratio MAOC/SOC is negatively related to sward age. In other words, long-aged sward grasslands accumulate more POC , lowering the ratio MAOC:SOC. The table 5 reports only the absolute values.

135 This would be the alternate hypothesis to the one we present in hypothesis ii. Section 4.3, lines 310 to 330 have been updated, highlighted in yellow below, to include discussion of this alternative hypothesis, and the potential caveats due to small sample size of the 16 to 20 years age group.

“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 it appears that 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.”

160

Line 115: Is not clear if the comparison of MAOC across sites treats the ‘site as random factor (One Random Factor ANOVA).

For the comparison of fine fraction OC across the sites (results displayed Figure 1, panel B) Kruskal test and post hoc Dunn testing was used as the data lacks homogenous variance and therefore does not meet the requirements of 165 an ANOVA. This is outlined in section 2.3.

Line 237-238. This statement does not explain the lower MAOC proportion in UK grassland compared to other ‘grassland’ sites. Was the MAOC separation method the same?

170 Under restructuring to clarify the focus of the manuscript as suggested by reviewer 2, this statement has been removed.

Table 3: please, add the r2 for completeness

Done.

175

Figure 2: please, add x (independent variable) in the equations

Done.

Response to Reviewer 2 Comments on Manuscript, bg-2020-273

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We thank the reviewers for their comments and evaluation of our manuscript. Please find below our response to
comments made by reviewer 2. Reviewer's comments are in black text, and our responses are in blue text,
changes to the manuscript are highlighted in yellow. Line numbers refer to the revised manuscript (marked
10 version) below.

Overall Comments:

The authors present an interesting dataset and thoughts. However, it is not so clear what the take home messages
are: The influence of soil types on <20 m OC? One time physical disturbance might not play a major role for <20
15 m OC? The evaluation of other unifactorial empirical linear models? In its current form the manuscript contains a
slightly confusing mixture. Based on the interesting data I suggest to do a major shift and set a new focus beyond
unifactorial models. In Table 2 there are so many highly significant correlations between various factors. These
should be explored further and related with carbon stocks to develop a higher potential of the manuscript and
advance insights into potential processes that govern soil organic carbon stocks in UK grasslands.

20 We thank the reviewer for the comments and have made revisions to the manuscript in order to clarify the take
home messages. The introduction has been restructured to provide sufficient background information of the aims
and objectives. The concluding paragraph has been amended to focus on the key take away points with respect to
the hypotheses outlined in the introduction. We agree that correlations between the variables presented are
interesting, and the suggested exploration would provide further insights into carbon stocks in UK grasslands.

25 The recommendation as described has been carried out as part of a sister project that focusses on soil C stocks. A manuscript based on that work is currently being prepared. Therefore, the aim of this separate study was to take a subset of samples to examine the suitability of the Hassink, (1997) linear regression equation, and to explore methods of estimating maximum fine fraction OC, and the impact of reseeding events on current fine fraction OC and predicted maximums. Taking the reviewer's advice on board, we believe that the new structure better frames
30 the aims and objectives and take home messages.

Specific Comments:

- As described in line 98, the so-called mineral-associated OC 'MAOC' is isolated by sieving. The authors should provide evidence of mineral association or reword this.
35 Whilst the mineral associated organic matter fraction, and C within, i.e. MAOC, has been defined to refer to just OM adsorbed to minerals, or include OM encapsulated in micro-aggregates (Kögel-Knabner et al., 2008), resulting in size based definitions ranging from 0 to 20 μm or 0 to 53 μm (Lavalée et al., 2020; Six et al., 2002). The term "OC in the fine fraction (< 20 μm)" has been used throughout as it was not possible to provide empirical evidence of mineral association, and also to overcome assumptions of fraction size which may be
40 associated with the term MAOC. The title has also been amended to reflect this change to "Estimating maximum fine fraction organic carbon in UK grasslands"

- As authors write themselves in line 200 the soil properties vary beyond only the proportion of fine fraction, this should be explored further as stated above. In Fig. 4 it seems that the relationship exists only across sites but not
45 within sites.

The following has been added to line 251 to 253.

"The range of reported values, and differences across the UK sites (Tables A3 and A4), suggests that the effect of the proportion of fine fraction of a sample on fine fraction OC is not consistent and likely reflects differences in

50 pedogenic and environmental conditions, and land management. It may be that the use of the mass proportion 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.”

55 - The use of abbreviations sometimes makes the reading hard; this should be minimized as much as possible (soil
type names for example).

This has been addressed throughout the text. Where abbreviations are used in tables or figures they have been
clearly defined in the captions.

60 - As described in line 247, the high variability of the proportion of the fine fraction might seriously confound the
analysis of sward age. Is it possible to correct for this using a multifactorial model?

65 The reviewer makes an interesting suggestion and the proportion of fine fraction was indeed variable within and
among the different sward age groups. We tried to investigate this further by means of ANCOVA testing,
however as the data does not satisfy the assumptions the use of a robust ANCOVA. The package available in R
(WRS2) to do this only allows for two levels within the grouping variable (Mair and Wilcox, 2020). In order to
acknowledge potential confounding effect, on variables linked to mass proportion of the fine fraction, the
following has been added to the discussion, lines 326 to 329.

70 “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.”

75 Line 15: Introduction very general, should be more specific towards the research questions

The introduction has been edited to provide more focused background to the objectives of the study, with a brief background to carbon sequestration, methods of predicting maximum fine fraction OC, (objectives i and ii), and the effects of grassland re-seeding events, (objective iii). See updated manuscript.

80 Line 19: What are specific challenges with this?

The following has been added to line 66 to elaborate this point in the introduction.

“There is a need for validity checks to determine the suitability of the Hassink, (1997) linear regression equation to predict maximum fine fraction OC of the soils in the respective studies. Without this sequestration potentials may be both over and underestimated.”

85

The following has been added to the discussion to clarify the issues, lines 231 to 234.

“However, the Hassink (1997) linear regression equation, equation has been used to estimate sequestration potentials, without prior testing to determine its applicability to the soils in question (e.g. Angers et al., 2011;

90 Chen et al., 2019; Lilly and Baggaley, 2013; Wiesmeier et al., 2014). This may have potentially over or underestimated sequestration potential, which may have repercussions for decisions made regarding land management”

Line 46: What is the specific soil management involved?

95 Details have been added to line 70.

“The high levels of disturbance associated with re-seeding events by mould board ploughing and harrowing in particular, result in changes in soil structure, notably the breaking up of aggregates, nutrient cycling and SOC mineralisation (Carolan and Fornara, 2016; Drewer et al., 2017; Soussana et al., 2004).”

100 Line 48: Other forms of OC would be probably also mineralized, why especially ‘MAOC’?

We agree that other forms of OC will also become more accessible for mineralisation due to the disturbance associated with reseeding events. However, as the focus of this work is on the mineral associated pool, we highlight that the disruption of aggregates in particular makes MAOC more accessible for microbial mineralisation. The following amendments have been made to better emphasize this and how the disturbance of other forms of OC may cause MAOC mineralisation, lines 75 to 80.

105

“Organo-mineral associations form the basis of microaggregates (Baldock and Skjemstad, 2000), and thus the destruction of aggregates makes the organo-mineral stabilised OC in the fine fraction, more accessible for mineralisation by the soil microbial community. Additionally, the release of other organic carbon pools may 110 induce a priming effect, potentially enhancing the losses from the typically stable mineral associated OC in the fine fraction. 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 thus the SOC sequestration ability of managed grasslands.”

115 Line 51: Please add that such estimation of a ‘protective capacity’ is empirical

Done.

Line 143: One decimal would probably be sufficient

Done.

120

Line 187: “very little further testing [: : :] in other soils” There are many papers cited in the manuscript that do exactly this to my understanding

The meaning here is in reference to studies which used the linear equation (e.g. Angers et al., 2011; Chen et al., 2019; Lilly and Baggaley, 2013; Wiesmeier et al., 2014) without assessing its applicability to the specific soils in question. It is not intended to refer to studies which have tried to improve the method of estimating maximum fine fraction OC. The following changes have been made, lines 231 to 234.

125 “However, the Hassink (1997) linear regression equation has been used to estimate sequestration potentials, without prior testing to determine its applicability to the soils in question (e.g. Angers et al., 2011; Chen et al., 130 2019; Lilly and Baggaley, 2013; Wiesmeier et al., 2014). This may have potentially over or underestimated sequestration potential, which may have repercussions for decisions made regarding land management.”

Line 194: Such validity tests probably would include grassland soils which might not always be available (as paired site), also given that there are many other specific factors – what do you specifically propose?

135 Given the results of the study, if someone wished to try to estimate maximum fine fraction OC using a univariate regression method, we suggest they develop their own linear regression equation by determining OC content of the fine fraction and mass proportion of fine fraction of a subset of the sample. Rather than assuming that the Hassink, (1997) is valid for their soils, see line 243.

140 Line 214: Interesting paragraph but far apart from the data presented here, what can you conclude and contribute to the discussion based on the data in this manuscript?

The paragraph has been amended to provide a better discussion focused on the results from this study. Lines 283 to 294.

145 Line 223: Add reference of original measurements

Done.

Line 225: I disagree with such simplified relation. Fungal origin should be verified with another biomarker approach. High C:N could result from root input and particulate OM (as briefly mentioned in line 238). Could 150 C:N results be influenced by mineral N fertilizer?

Under the restructuring of the manuscript this comment no longer applies.

Line 237: What could this “other means” be?

Lines 331 to 333, have been amended to the following;

155

“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.”

160 Line 255: Could you add a literature reference here?

Done.

Line 445, Figure 1: The data is repeated in the Appendix. Should be present only at one spot.

165 The data in the appendix gives readers the opportunity to examine the results between the fields within each site, which is not possible from figure 1, so, respectfully, we suggest to retain both.

Line 455, Figure 2: To improve the comparison between panels, I suggest to put similar x and y scales. Also add the significance level and remove regressions from the Figure when not significant.

The x and y-axis have been adjusted and significance levels added. However, regression details have been retain

170 for transparency.

References:

Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K. and Leinweber, P.: Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry, *J. Plant Nutr. Soil Sci.*, 171(1), 61–82, doi:10.1002/jpln.200700048, 2008.

175 Lavallee, J. M., Soong, J. L. and Cotrufo, M. F.: Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century, *Glob. Chang. Biol.*, 26(1), 261–273, doi:10.1111/gcb.14859, 2020.

Mair, P. and Wilcox, R.: Robust statistical methods in R using the WRS2 package, *Behav. Res. Methods*, 52(2),

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Six, J., Conant, R. T., Paul, E. A. and Paustian, K.: Stabilization mechanisms of SOM implications for C saturation of soils, *Plant Soil*, 241(2), 155–176, doi:10.1023/A:1016125726789, 2002.

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Estimating maximum fine fraction ~~mineral associated~~ organic carbon in UK grasslands

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15 **Abstract.** Soil organic carbon (SOC) sequestration across agroecosystems worldwide can contribute to mitigate the effects of climate change by reducing levels of atmospheric CO₂. Stabilisation of organic carbon (OC) in the fine soil fraction (< 20 μm)~~Mineral associated organic carbon (MAOC)~~ is considered an important long-term store of SOC and the saturation deficit (difference between measured MAOC and estimated maximum MAOC 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 (BL) analysis and quantile regression (QR) 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 MAOC in UK grassland soils of varying sward ages and to evaluate the linear regression, boundary line~~BL~~ and quantile regression~~QR~~ methods to estimate maximum fine fraction OCMAOC. A chronosequence of 10 grasslands was sampled, in order to assess the relationship between sward age (time since last reseeding event) and the measured~~current~~ and predicted maximum fine fraction OCMAOC. Significantly different regression equations show that the Hassink, (1997) equation does not accurately reflect maximum fine fraction OC MAOC in UK grasslands when determined using the proportion of fine soil fraction (< 20 μm , %) and measured fine fraction OC (g C kg⁻¹ soil)~~current MAOC~~. 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 OCMAOC, Fine fraction OCMAOC, MAOC 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

~~finemineral~~ fraction of these soils, or stabilisation in aggregates is predominant in these grassland soils. This work highlights the need to ensure that methods used to predict maximum ~~fine fraction OCMAOC~~ 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 CO₂ 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). ~~However, to utilise soils as a CO₂ drawdown mechanism, accurate estimates of their storage capability are required. Carbon sequestration refers to the removal of CO₂ from the atmosphere into long lived soil C pools, which would not otherwise occur under current management practices (Lal, 2004; Powlson et al., 2011). Soil organic carbon (SOC) is stabilised by three mechanisms i) inherent chemical recalcitrance, ii) adsorption to mineral surfaces, and iii) occlusion of SOC within soil aggregates. With respect to soil organic carbon (SOC) sequestration, organic carbon (OC) stabilised via adsorption to mineral surfaces the mineral associated organic carbon (MAOC) 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, as sample size is not reduced as in BL 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). These suggestions have been

proposed to improve estimates of maximum fine fraction OC. However, several studies use the original equation presented by Hassink, (1997) to estimate sequestration potential at different scales (e.g. Angers et al., 2011; Chen et al., 2019; Lilly and Baggaley, 2013; Wiesmeier et al., 2014). There is a need for validity checks to determine the suitability of the Hassink, (1997) linear regression equation to predict maximum fine fraction OC of the soils in the respective studies. Without this sequestration potentials may be both over and underestimated.

Within the UK, human-managed grasslands are the dominant land use in the UK, 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~~ They are thought to have high levels of disturbance associated with re-seeding events by mould board ploughing and harrowing in particular, result in changes in soil structure, notably the breaking up of aggregates, nutrient cycling and SOC mineralisation (Carolan and Fornara, 2016; Drewer et al., 2017; Soussana et al., 2004). They are thought to have high potential for sequestering more C potential for sequestering more C (Smith, 2014), however frequent re-seeding may 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 (Balock and Skjemstad, 2000), and thus the destruction of aggregates makes the organo-mineral stabilised OC in the fine fraction, more accessible for mineralisation by the soil microbial community. Additionally, the release of other organic carbon pools may induce a priming effect, potentially enhancing the losses from the typically stable mineral associated OC in the fine fraction. 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 thus the SOC sequestration ability of managed grasslands. The long term effect of re-seeding on SOC is understudied but is likely to affect physical soil aggregates making MAOC accessible for microbial mineralisation, and enhance the potential for SOC losses. It is therefore important to understand how disturbance might affect MAOC and thus the SOC sequestration ability of managed grasslands.

To utilise soils as a CO_2 drawdown mechanism, accurate estimates of their storage capability are required. It is well accepted that there is an upper protective capacity limit, or saturation point of MAOC (Six et al., 2002; Stewart et al., 2007). The ability to predict this saturation point is essential in order to assess the feasibility of SOC sequestration targets. Hassink, (1997) compared pairs of Dutch arable and grassland soils and found that while soil bulk SOC contents significantly differed among soils, MAOC did not. A positive relationship between the mass proportion of the fine soil fraction and associated C and N concentrations in temperate and tropical soils was also observed. 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 MAOC (g kg^{-1} soil). With this approach, potential SOC sequestration (or saturation deficit) can be estimated by subtracting the current MAOC from the estimated maximum MAOC (MAOC_{max}) (Angers et al., 2011). Several iterations of the concept have been proposed to overcome the limitations of linear regression. For example, boundary line analysis (BL) uses a defined upper or lower subset of a data set to estimate the boundary line, when a limiting response to

95 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, BL 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 (QR) 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 $MAOC_{max}$, as sample size is not reduced as in BL 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 $MAOC$ without any fine soil fraction (Beare et al., 2014; Feng et al., 2013; Liang et al., 2009). These suggestions have been proposed to improve estimates of $MAOC_{max}$. However, several 100 studies use the original equation presented by Hassink, (1997) to estimate sequestration potential at different scales (e.g. Angers et al., 2011; Chen et al., 2019; Lilly and Baggaley, 2013; Wiesmeier et al., 2014). This is frequently done without any validation 105 checks to determine the suitability of the Hassink, (1997) linear regression equation to predict $MAOC_{max}$ in the respective studies. This may lead to both over and underestimations of sequestration potential, which may have repercussions for decisions made regarding grassland management.

110 The objectives of this study were (i) to assess the suitability of the Hassink, (1997) equation to estimate maximum fine fraction $OCMAOC_{max}$ in UK grassland soils of varying sward ages, (ii) to evaluate the linear regression, boundary lineBL and quantile regressionQR methods to estimate maximum fine fraction OC MAOC_{max}, and (iii) to explore the relationship between sward age (time since last reseeding event), and current and predicted maximum- fine fraction OC MAOC. We hypothesised that i) 115 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, would have a greater proportion of total SOC stabilised in- fine fraction (< 20 μm) as MAOC and a lower sequestration potential.

2. Materials and Methods

2.1 Site Description and Sampling

120 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 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

125 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
130 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
135 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 20°C and 35°C (Jackson, 2005). After the
140 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 ~~MAOC~~ 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,
145 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.

150 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 MAOC_{max} with the mass proportion of fine fraction (< 20 μm , %) in a sample and the measured MAOC 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 160 regression with a forced zero intercept was used with data from this study and the data published in Hassink (1997).

Boundary lineBL analyses were performed as an alternative to linear regression, both with and without a forced zero intercept to predict maximum fine fraction OCMAOC_{max} 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 MAOC_{max}maximum fine fraction OC. The groups were then ordered by measured fine fraction OCMAOC (g C kg^{-1} soil), and the values in the 90th percentile were used to plot the boundary line. Boundary lineBL analysis was not used for individual sites as it resulted in too few data points. Quantile regressionQR 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 165 170 ‘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 in order to identify the degree of saturation across the sites, when estimating maximum fine fraction OCMAOC_{max} using the Hassink (1997), UK, and site-specific linear regression equations both with 175 and without a forced zero intercept, and the equations generated by boundary lineBL and quantile regressionQR analyses. The carbon saturation ratio was calculated by dividing the current fine fraction OCMAOC by the estimated maximum fine fraction OC contentMAOC_{max}. Values < 1 were deemed under saturated, = 1 as at saturation and > 1 as oversaturated.

3. Results

180 3.1 Current C concentrations

The measured total SOC and fine fraction OCMAOC concentrations exhibited variation within the grassland sites (Fig. 1). Total SOC varied from 8.2 to 85.84 g C kg^{-1} soil, with a median of 32.72 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 MAOC ranged from 1.437 to 20.989 g C kg⁻¹ soil, with a median of 6.24 g C kg⁻¹ soil. Overton had the highest 185 total fine fraction OC MAOC ($P < 0.05$) and was the only organically managed site (Fig. 1). The proportion of OC stabilised in the stored fine fraction (< 20 µm) as MAOC had high variability across the UK sites accounting for 4.5 to 50.12% of total SOC with a median of 17.54%. The proportion of total SOC stabilised in the fine fraction (< 20 µm) stored as MAOC, and proportion of fine fraction in a sample did not significantly differ in Harpenden and Overton, however they have 190 significantly different measured current fine fraction OC content current MAOC (g C kg⁻¹ soil) ($P < 0.05$), indicating different saturation potentials (Fig. 4). 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 C:N ratio was significantly different between the sites, Figure 1, however the mean of all the data showed little deviation, 9.84 ± 1.00 (mean \pm standard deviation). Full details of all the measured properties of bulk and fine fraction, per field are presented in Table A1.

195 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 current MAOC 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) MAOC 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), MAOC to SOC_{total} was greatest in luvisols ($P < 0.05$) (Fig. 3).

3.12 Estimated maximum fine fraction organic carbon MAOC

205 The slope generated from the UK data used to estimate MAOC (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, 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).

210 Coefficients from BL 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 QR analysis had a similar slope to the BL boundary line and linear regression with forced zero intercept. QR quantile regression QR 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^{QR}, 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.

3.23 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 μ m), current MAOC (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 the individual sites, significant differences were observed between fields, with some properties, but again there was no consistent effect of sward age (Tables A3 and A4).

4. Discussion

4.1 Estimation of maximum mineral associatedfine 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 fraction OC contentMAOC is an established method with a strong grounding in correlation between the variables. However, despite the wide use of the Hassink (1997) linear regression equation has been used to estimate sequestration potentials, without prior, it has undergone very little further testing to determine its applicability to the soils in question (e.g. Angers et al., 2011; Chen et al., 2019; Lilly and Baggaley, 2013; Wiesmeier et al., 2014) suitability in other soils. This may have potentially over or underestimated sequestration potential, which may have repercussions for decisions made regarding land management. The significantly different slopes for the linear regression equations (Table 3) shows that the Hassink (1997) regression equation is not suitable for estimating maximum fine fraction OCMAOC_{max} in UK grasslands. Previous concerns have focused on the potential for the equation developed by Hassink (1997) to underestimate maximum fine fraction OCMAOC_{max}, as 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 OCMAOC_{max} using the Hassink (1997) regression approach resulted in a significant overestimation of fine fraction OCMAOC_{max} sequestration potential. Future work using maximum fine fraction OCMAOC_{max} predictions equations reported in the literature (e.g. Beare et al., 2014; Feng et al., 2013; Hassink, 1997; Six et al., 2002) (e.g. Beare et al., 2014; Feng et al., 2013; 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.

245 To overcome the contradiction of an intercept greater than zero, indicating that C is stabilised in the fine fractions MAOC without any fine fraction, a forced zero intercept was used. The linear regression slopes with a forced zero intercept were not 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 250 proportion of fine fraction of a sample on MAOC fine fraction OC is not consistent and likely reflects differences in pedogenic and environmental conditions, and land management and possibly the fine fraction OC isolation method. It may be that the use of the mass proportion 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.

255 Boundary line (BL)-analysis and quantile regression (QR) have been suggested as alternatives to overcome the limitations of linear regression. The estimation of maximum fine fraction OC MAOC_{max} was greatest when using quantile regression QR ($\tau = 0.90$), whereas boundary line BL estimates at 5 and 10% intervals were similar to quantile regression QR ($\tau = 0.50$), and those estimated from linear regression (Table 3). The use of the median percentile quantile regression QR highlights the closeness of linear regression predictions being more indicative of mean values, thus underestimating SOC 260 sequestration potential. The boundary line BL 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 BL 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 MAOC 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 C sequestration estimate generated by boundary line BL analysis is biased by the range of data.

265 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. Therefore, 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 270 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. However, this was not the case at the site level (Fig. A1). Where 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.

275 Therefore, despite a higher fine fraction OC contents 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 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
280 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.

285 Estimating maximum fine fraction OC on the basis of 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
290 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 nitrogen 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.

295 The influence of soil type of 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
300 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 identify where fine fraction OC needs to be protected, but also where it can be enhanced.

305 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 which 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. In particular further
empirical evidence of how to manipulate fine fraction OC stabilisation processes in a way that is practical for grassland

management to promote the formation of new organo-mineral associations, and understanding their stability will be important for establishing the true potential of additional carbon sequestration across managed grasslands.

4.3 Effect of sward age on fine fraction OCMAOC

310 It was anticipated that for fields of an older sward age, a greater proportion of total SOC would be MAOC stabilised as fine fraction OC, as tillage breaks up macroaggregates making OC in the fine fraction MAOC 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. However, the proportion of total SOC stabilised in the fine fraction that is MAOC was not consistently higher

315 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 MAOC, the proportion of fine fraction in a sample (%) by mass, and measured fine fraction OCMAOC (g C kg⁻¹ soil) and the relative proportion of measured fine fraction MAOC of the total SOC content of the bulk soil (MAOC (% of SOC_{total})), i.e MAOC:SOC ratio 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 320 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 it appears that 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 325 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 330 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 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. The high density of fine roots contributing to aggregate formation suggests physical protection is likely 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 ($> 2000 \mu\text{m}$, $250\text{--}2000 \mu\text{m}$, $53\text{--}250 \mu\text{m}$ and $< 53 \mu\text{m}$) (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 (DOC) from below ground inputs is more efficiently stabilised in organo-mineral associations as MAOC than above ground dissolved organic carbon (DOC) (litter leachate) (Sokol and Bradford, 2019). The narrow rhizosphere to bulk soil ratio in grasslands, means that this below ground pathway is of greater importance for both total SOC and MAOC (Sokol and Bradford, 2019). This may make the fine fraction MAOC 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 MAOC in a range of UK grassland soils. The significantly different slopes across the UK demonstrate the variability of the effect of proportion of fine fraction in a sample and current MAOC. Therefore, caution should be applied to estimates of maximum fine fraction OC MAOC obtained using the Hassink (1997) equation, in instances where it may not accurately reflect fine fraction OC MAOC stabilisation processes 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 linear regression. If estimating maximum MAOC using the proportion of fine fraction and current MAOC, the use of QR at the 90th percentile is recommended to overcome shortfalls of linear regression. However, such a simple estimate is unlikely to accurately reflect the dynamics of fine fraction OC MAOC stabilisation. This work has helped to identify some key parameters which 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 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. and additional research is required to elucidate parameters which balance resource inputs and predictive power. Such work would help to accurately assess the feasibility of achieving soil carbon sequestration targets. In temperate soils such as the UK grassland soils studied here, MAOC only made up a small proportion of total SOC suggesting a dominance of other stabilisation processes. Whilst there was an inconsistent effect of sward age in this study, further research to understand dominant SOC stabilisation and its resilience in response to land 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, data analysis, and prepared the manuscript draft. All authors contributed to editing and reviewing of the manuscript.

Data Availability

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

Competing Interest

The authors declare that they have no conflict of interest.

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480 **Table 1** Summary of UK grassland site characteristics.

| Site | Age range (years) | Land Use ^a | Mean Annual Temperature (°C) ^b | Mean Annual Rainfall (mm) ^a | Elevation (m.a.s.l) | WRB Type ^c | Soil | 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 loam | clay |
| 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 ($<20 \mu\text{m}$) soil properties, sward age and known environmental parameters.

| | | | | Bulk Soil | | | | | | Fine Fraction | | | |
|---------------|-----------------------------|----------|----------|-----------|----------------|----------------|---------|----------|----------------|-----------------------------|----------------|----------------|-----|
| | | Temp. | Prec. | Age | %N | %C | C:N | pH | %SC | <u>Fine fraction OCMAOC</u> | %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 | | | | | |
| Fine Fraction | %SC | 0.26*** | -0.43*** | 0.14* | 0.12 | 0.12 | -0.01 | 0.21*** | 1 | | | | |
| | <u>Fine fraction OCMAOC</u> | 0.13* | -0.36*** | 0.1 | 0.26*** | 0.27*** | 0.01 | 0.12 | - ^a | 1 | | | |
| | %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 |

^a No correlation calculated as one variable used to calculate the other.

490 Age; years since last reseeding event, Temp; median value from the mean annual temperature range ($^{\circ}\text{C}$), Prec.; mean annual rainfall (mm), %SC; mass proportion of fine fraction in a sample (%), Fine fraction OCMAOC; measured fine fraction OCMAOC; measured mineral associated organic carbon (g MAOC 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 (max MAOC) by linear regression (LR), linear regression with forced zero intercept (LR_0), boundary line (BL) and quantile regression (QR). Lettering indicates slopes which were significantly different within a method ($P < 0.05$).

| Method | | Slope (± 1 SEM) | P slope | Intercept (± 1 SEM) | P intercept | RMSE | n | R ² |
|--------|------------------------------|-------------------------------|---------|-----------------------------|----------------|------|-----|----------------|
| LR | Hassink, (1997) | 0.37 ^a | | 4.07 | | | 40 | |
| | All UK | 0.32 \pm 0.023 ^b | *** | 2.86 \pm 0.368 | *** | 2.58 | 129 | 0.61 |
| LR_0 | Hassink, (1997) ^a | 0.45 \pm 0.02 | *** | | | 4.97 | 40 | 0.94 |
| | All UK | 0.47 \pm 0.017 | *** | | | 3.13 | 129 | 0.85 |
| BL | 5% intervals | 0.48 \pm 0.058 | *** | | | 5.89 | 19 | 0.79 |
| | 10% intervals | 0.48 \pm 0.070 | *** | | | 6.36 | 15 | 0.77 |
| | 15% intervals | 0.56 \pm 0.056 | *** | | | 4.77 | 14 | 0.89 |
| QR | QR ($\tau = 0.90$) | 0.92 \pm 0.071 | *** | | | 7.90 | 129 | 0.90 |
| | QR ($\tau = 0.50$) | 0.49 \pm 0.032 | *** | | | 3.15 | 129 | 0.66 |

RMSE, root mean square error.

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

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

Table 4. Carbon saturation ratios calculated from the estimated maximum fine fraction organic carbon MAOC_{max} 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 | 50 th | 38 | 1.4 | 1.32 |
| | 90 th | 99 | 0.74 | 0.7 |

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530 **Table 5. Effect of sward age grouped at five year intervals on selected soil properties. Values are means \pm 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 MAOC (g C kg ⁻¹ soil) | Fine fraction organic carbon (% of SOC _{total}) |
|----------|----|-------------------------------|--------------------------------|---|---|
| 0 to 5 | 48 | 10.18 \pm 0.15 ^a | 10.00 \pm 1.41 ^a | 5.68 \pm 0.49 ^a | 18.32 \pm 1.5 ^{ab} |
| 6 to 10 | 18 | 9.79 \pm 0.26 ^{ab} | 14.47 \pm 1.69 ^b | 8.58 \pm 0.59 ^b | 24.94 \pm 1.35 ^c |
| 11 to 15 | 15 | 9.33 \pm 0.11 ^b | 15.27 \pm 2.98 ^{ab} | 9.17 \pm 1.66 ^{ab} | 20.66 \pm 2.55 ^{abc} |
| 16 to 20 | 9 | 10.41 \pm 0.31 ^a | 6.10 \pm 0.77 ^a | 4.68 \pm 0.44 ^a | 11.54 \pm 1.12 ^a |
| 21+ | 39 | 9.50 \pm 0.11 ^b | 15.19 \pm 1.69 ^b | 7.44 \pm 0.69 ^{ab} | 23.21 \pm 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.

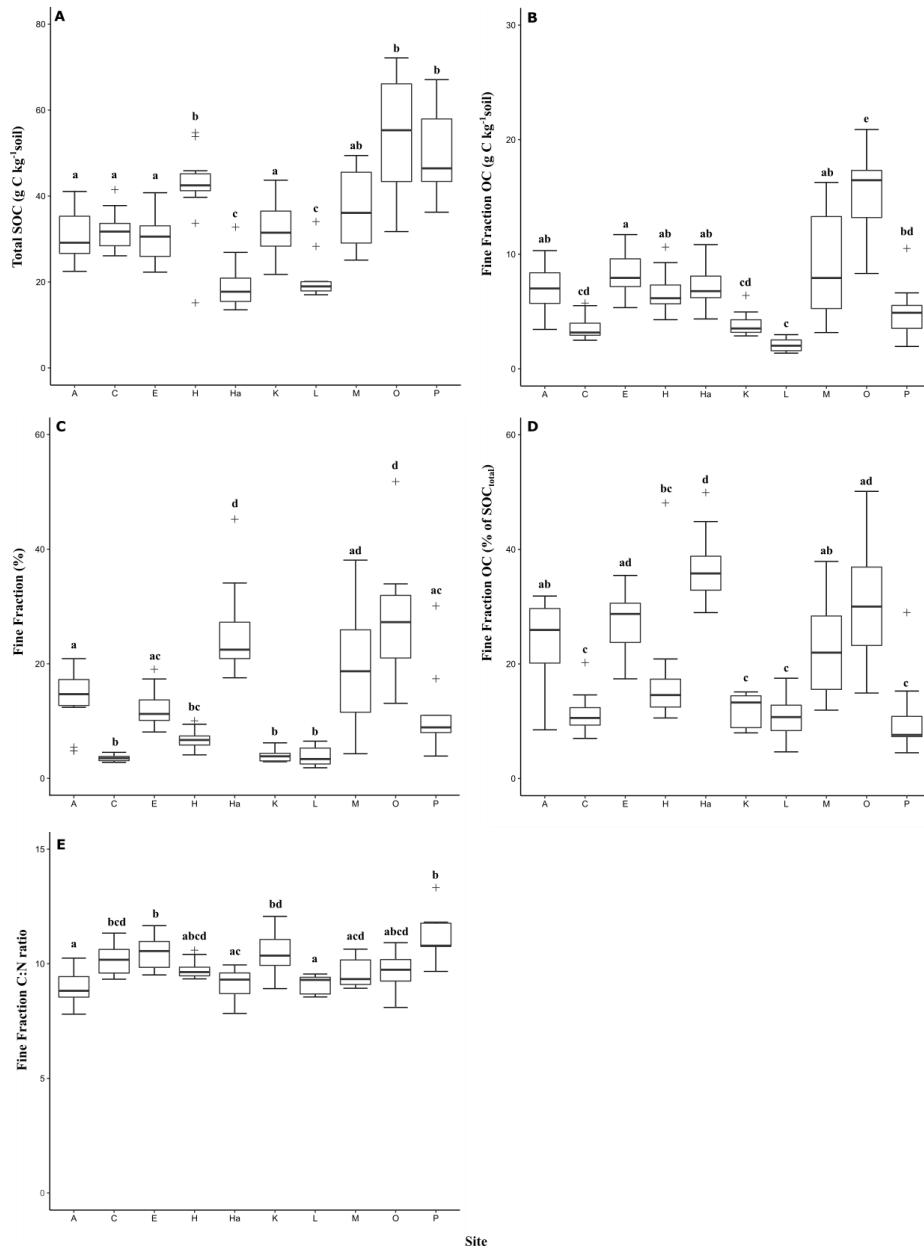
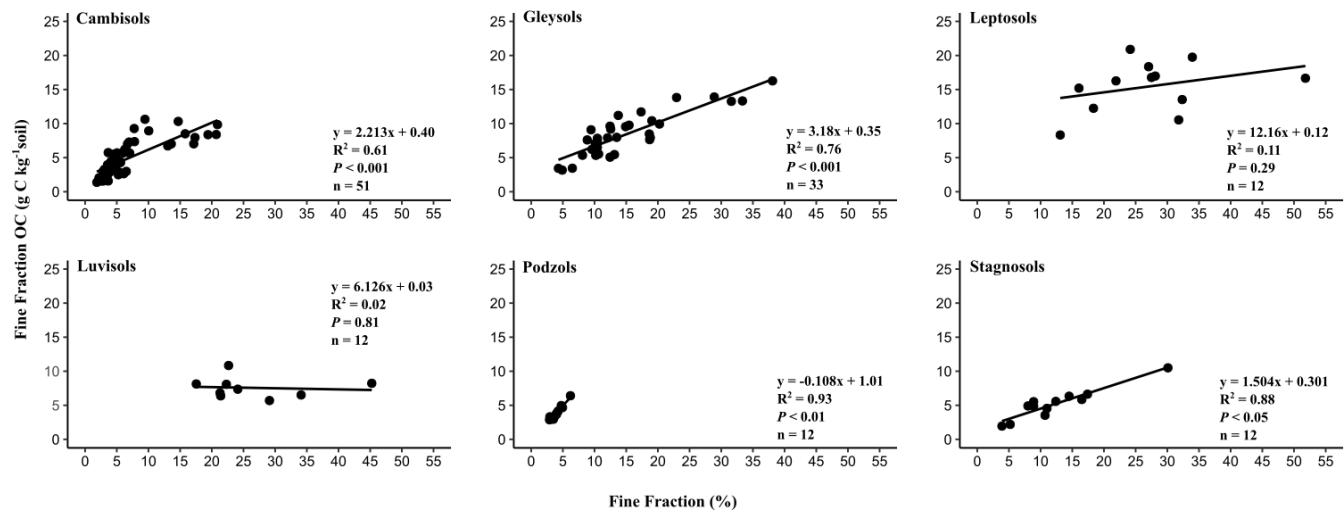


Figure 1. Measured total SOC ($\text{g C kg}^{-1}\text{soil}$) (A) total fine fraction organic carbon MAOC ($\text{g C kg}^{-1}\text{soil}$) (B), mass proportion of fine fraction ($< 20 \mu\text{m}$, %) (C), and relative proportion of measured fine fraction organic carbon current MAOC of the total SOC content of the bulk soil (D) and fine fraction C:N ratio (E), for each of the grassland sites; Aberystwyth (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 indicated as crosses (> 1.5 times the interquartile range). Lettering indicates significant differences between soils ($P < 0.05$).

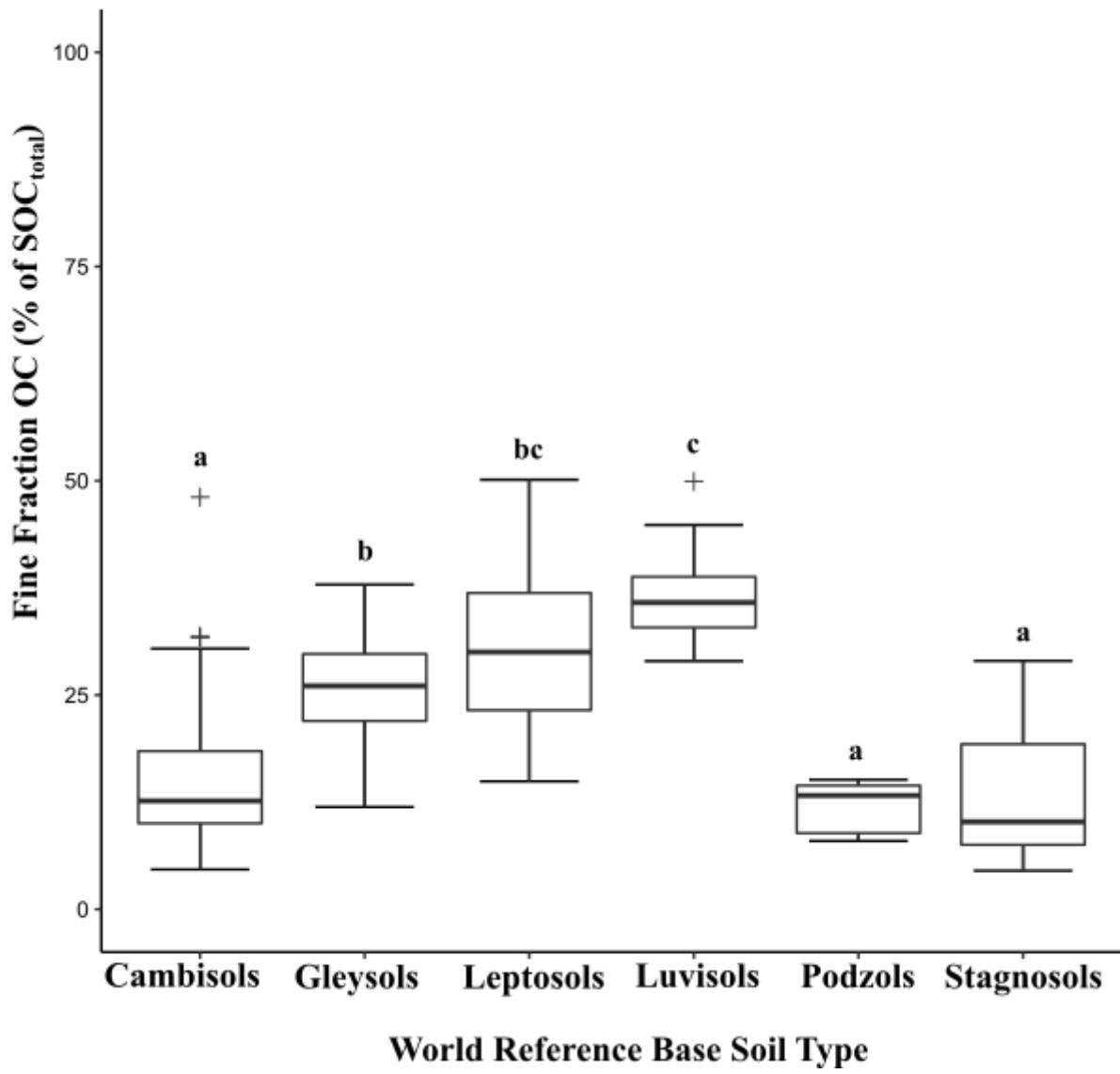


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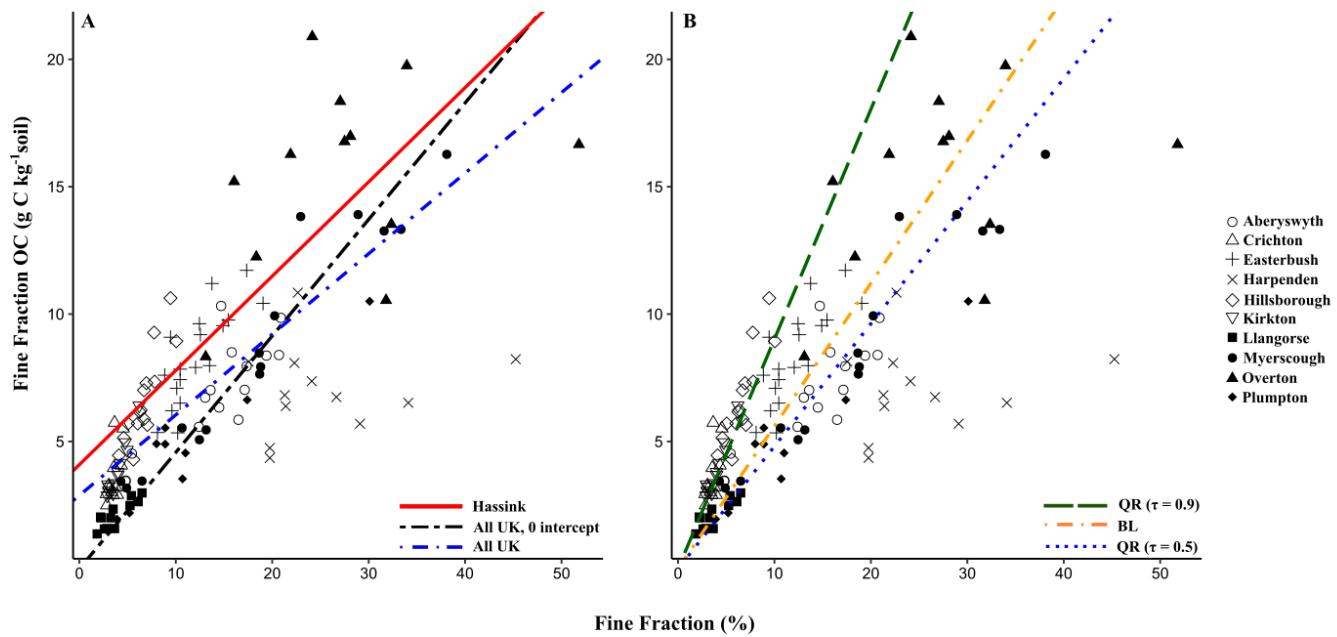
Figure 2. Relationships between mass proportion of the fine fraction (%) and fine fraction organic carbon-MAOC (g C kg⁻¹ soil) in the soil types used in this study; cambisols (CM), gleysols (GL), leptosols (LP), luvisols (LV), podzols (PZ) and stagnosols (ST). Asterisks indicate significance at $P < 0.05$.

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560 Figure 3. Relative proportion of measured **measured fine fraction organic carbon current MAOC** of the total SOC content of the
 bulk soil for the different soil types used in this study: **cambisols (CM), gleysols (GL), leptosols (LP), luvisols (LV), podzols (PZ)**
and stagnosols (ST). Lettering indicates significant differences at $P < 0.05$.



|570 **Figure 4.** Measured **fine fraction organic carbon MAOC** (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.

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Appendix A

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

| Site | Age | | pH | C:N | C (g C kg ⁻¹ soil) | C stock (t C ha ⁻¹) | N stock (t N ha ⁻¹) |
|--------------|-------------|--------------------------------|-------------------------------|--------------------------------|----------------------------------|------------------------------------|------------------------------------|
| | (year s) | BD ^a | | | | | |
| Aberystwyth | 2 | 1 \pm 0.01 ^a | 5.20 \pm 0.05 ^a | 9.70 \pm 0.05 ^b | 26.95 \pm 0.63 ^b | 73.61 \pm 1.73 ^b | 7.59 \pm 0.16 ^b |
| | 6 | 0.98 \pm 0.04 ^a | 4.70 \pm 0.04 ^{bc} | 9.68 \pm 0.08 ^b | 26.7 \pm 0.82 ^b | 73.86 \pm 2.28 ^b | 7.62 \pm 0.18 ^b |
| | 11 | 0.82 \pm 0.05 ^b | 5.12 \pm 0.06 ^a | 10.46 \pm 0.09 ^a | 29.72 \pm 0.83 ^b | 76.97 \pm 2.15 ^b | 7.36 \pm 0.20 ^b |
| | 31 | 0.74 \pm 0.05 ^b | 4.99 \pm 0.09 ^{ab} | 10.54 \pm 0.22 ^a | 29.4 \pm 1.63 ^b | 74.23 \pm 4.12 ^b | 7.01 \pm 0.31 ^b |
| | 33 | 0.69 \pm 0.03 ^b | 4.18 \pm 0.02 ^c | 10.59 \pm 0.10 ^a | 38.19 \pm 1.97 ^b | 95.67 \pm 4.92 ^a | 9.01 \pm 0.40 ^a |
| Crichton | 1 | 0.92 \pm 0.03 | 5.14 \pm 0.03 ^{ab} | 12.19 \pm 0.08 ^{ab} | 34.66 \pm 0.66 ^a | 82.40 \pm 1.56 | 6.76 \pm 0.11 ^b |
| | 3 | 0.99 \pm 0.07 | 5.65 \pm 0.06 ^b | 11.73 \pm 0.11 ^{bc} | 29.94 \pm 1.37 ^{ab} | 74.63 \pm 3.41 | 6.36 \pm 0.26 ^b |
| | 15 | 0.93 \pm 0.05 | 4.77 \pm 0.04 ^{ac} | 9.90 \pm 0.88 ^c | 30.85 \pm 3.06 ^b | 79.73 \pm 7.91 | 7.98 \pm 0.23 ^a |
| | 20 | 0.93 \pm 0.04 | 4.54 \pm 0.03 ^c | 13.21 \pm 0.14 ^a | 27.26 \pm 0.87 ^b | 66.62 \pm 2.11 | 5.04 \pm 0.15 ^c |
| Easter Bush | 3 | 1.02 \pm 0.04 ^{abc} | 5.45 \pm 0.06 ^{ab} | 13.03 \pm 0.11 ^{bc} | 32.46 \pm 1.29 ^a | 93.52 \pm 3.72 ^a | 7.17 \pm 0.26 ^a |
| | 5 | 1.19 \pm 0.03 ^a | 5.44 \pm 0.06 ^{ab} | 12.84 \pm 0.21 ^{bc} | 26.41 \pm 0.54 ^b | 74.45 \pm 1.52 ^b | 5.80 \pm 0.10 ^{bc} |
| | 5 | 0.84 \pm 0.06 ^c | 5.67 \pm 0.04 ^a | 11.74 \pm 0.17 ^d | 27.50 \pm 1.0 ^b | 58.15 \pm 2.12 ^c | 4.94 \pm 0.14 ^d |
| | 6 | 0.96 \pm 0.05 ^{bc} | 5.32 \pm 0.06 ^b | 12.45 \pm 0.13 ^c | 30.46 \pm 1.93 ^{ab} | 71.16 \pm 4.50 ^{bc} | 5.72 \pm 0.36 ^{cd} |
| | 6 | 1.12 \pm 0.05 ^{ab} | 5.81 \pm 0.20 ^a | 14.15 \pm 0.13 ^a | 28.95 \pm 0.99 ^{ab} | 75.69 \pm 2.59 ^b | 5.35 \pm 0.17 ^d |
| | 8 | 1.12 \pm 0.03 ^{ab} | 4.99 \pm 0.04 ^c | 13.43 \pm 0.11 ^b | 33.03 \pm 0.50 ^a | 89.43 \pm 1.34 ^a | 6.66 \pm 0.10 ^{ab} |
| Harpenden | 22 | 1.37 \pm 0.07 | 7.37 \pm 0.04 ^a | 12.09 \pm 0.2 | 16.06 \pm 0.59 ^c | 25.37 \pm 0.93 ^c | 3.3 \pm 0.12 ^c |
| | 68 | 1.12 \pm 0.08 | 5.85 \pm 0.12 ^{ab} | 12.34 \pm 0.08 | 19.8 \pm 0.63 ^b | 50.49 \pm 1.59 ^b | 4.06 \pm 0.13 ^b |
| | 179 | 1.09 \pm 0.14 | 5.63 \pm 0.06 ^b | 12.8 \pm 0.26 | 28.7 \pm 1.47 ^a | 72.98 \pm 3.74 ^a | 5.89 \pm 0.30 ^a |
| Hillsborough | 1 | 1.79 \pm 0.10 | 6.31 \pm 0.07 ^a | 11.25 \pm 0.12 ^{ab} | 46.68 \pm 2.04 | 120.16 \pm 5.26 ^{ab} | 10.69 \pm 0.51 ^{ab} |
| | 7 | 1.88 \pm 0.08 | 5.10 \pm 0.04 ^b | 11.46 \pm 0.11 ^b | 42.85 \pm 1.52 | 108.86 \pm 3.87 ^b | 9.51 \pm 0.34 ^{bc} |
| | 16 | 1.79 \pm 0.05 | 5.33 \pm 0.08 ^b | 10.87 \pm 0.06 ^c | 42.36 \pm 1.98 | 111.63 \pm 5.21 ^{ab} | 10.27 \pm 0.47 ^{ab} |
| | 23 | 1.75 \pm 0.05 | 4.76 \pm 0.03 ^c | 11.33 \pm 0.09 ^{ab} | 46.44 \pm 1.78 | 125.43 \pm 4.82 ^a | 11.08 \pm 0.45 ^a |
| | 37 | 1.69 \pm 0.06 | 5.13 \pm 0.06 ^b | 10.34 \pm 0.77 ^{ac} | 40.90 \pm 3.10 | 86.04 \pm 6.52 ^c | 8.38 \pm 0.24 ^c |
| Kirkton | 1 | 0.9 \pm 0.04 | 4.78 \pm 0.04 ^c | 12.13 \pm 0.11 ^c | 27.90 \pm 0.81 ^c | 82.03 \pm 2.39 ^b | 6.77 \pm 0.22 |
| | 3 | 0.95 \pm 0.04 | 5.49 \pm 0.06 ^a | 12.61 \pm 0.15 ^b | 36.67 \pm 1.56 ^a | 98.19 \pm 4.17 ^{ab} | 7.79 \pm 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 |
| Llangorse | 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 |
| | 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 ± 0.13 ^a | 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 |
| Plumpton | 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 |
| | 1 | 0.99 ± 0.02 ^a | 6.34 ± 0.08 ^b | 10.85 ± 0.08 ^{ab} | 40.92 ± 1.21 ^b | 122.21 ± 3.61 ^b | 11.26 ± 0.28 ^b |
| | 5 | 1.08 ± 0.03 ^a | 7.15 ± 0.06 ^a | 11.27 ± 0.41 ^a | 45.55 ± 0.61 ^b | 132.09 ± 1.77 ^b | 11.87 ± 0.48 ^b |
| | 20 | 0.72 ± 0.04 ^b | 5.38 ± 0.21 ^c | 10.54 ± 0.17 ^b | 58.08 ± 2.36 ^a | 163.23 ± 6.62 ^a | 15.47 ± 0.56 ^a |

^aBulk 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 which are significantly different, within a site ($P < 0.05$).

| Location | Age (years) | %N | %C | C:N | % <u>Fine</u> <u>Fraction</u> <u>SC</u> | <u>Organic</u> <u>Carbon</u> <u>MAOC</u> (g C kg ⁻¹ bulk soil) ^a | <u>Organic</u> <u>Carbon</u> <u>MAOC</u> —(% of SOC _{total}) |
|--------------|----------------|---------------------------|---------------------------|----------------------------|---|--|---|
| Aberystwyth | 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 |
| Llangorse | 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 |
| | 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**SC**; 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.

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

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

RMSE: Root mean square error.

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

|625 **Table A4. Linear regression coefficients for the estimation of maximum fine fraction organic carbon maximum MAOC (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 |
|---------------------|------------------------------------|-----|-------|----|----------------|
| Aberystwyth | 0.47 \pm 0.024 ^{bc} | *** | 1.357 | 15 | 0.96 |
| Crichton | 1.02 \pm 0.067 ^{cdef} | *** | 0.796 | 12 | 0.95 |
| Easter Bush | 0.67 \pm 0.024 ^e | *** | 1.231 | 18 | 0.98 |
| Harpenden | 0.26 \pm 0.035 ^a | *** | 2.739 | 9 | 0.87 |
| Hillsborough | 0.99 \pm 0.033 ^f | *** | 0.842 | 15 | 0.99 |
| Kirkton | 0.98 \pm 0.0197 ^{def} | *** | 0.265 | 12 | 0.99 |
| Llangorse | 0.52 \pm 0.035 ^{abcdef} | *** | 0.474 | 12 | 0.95 |
| Mysercough | 0.45 \pm 0.016 ^b | *** | 1.255 | 15 | 0.98 |
| Overton | 0.52 \pm 0.055 ^{bcd} | *** | 5.297 | 12 | 0.89 |
| Plumpton | 0.39 \pm 0.030 ^{ab} | *** | 1.141 | 9 | 0.96 |

RMSE: Root mean square error.

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

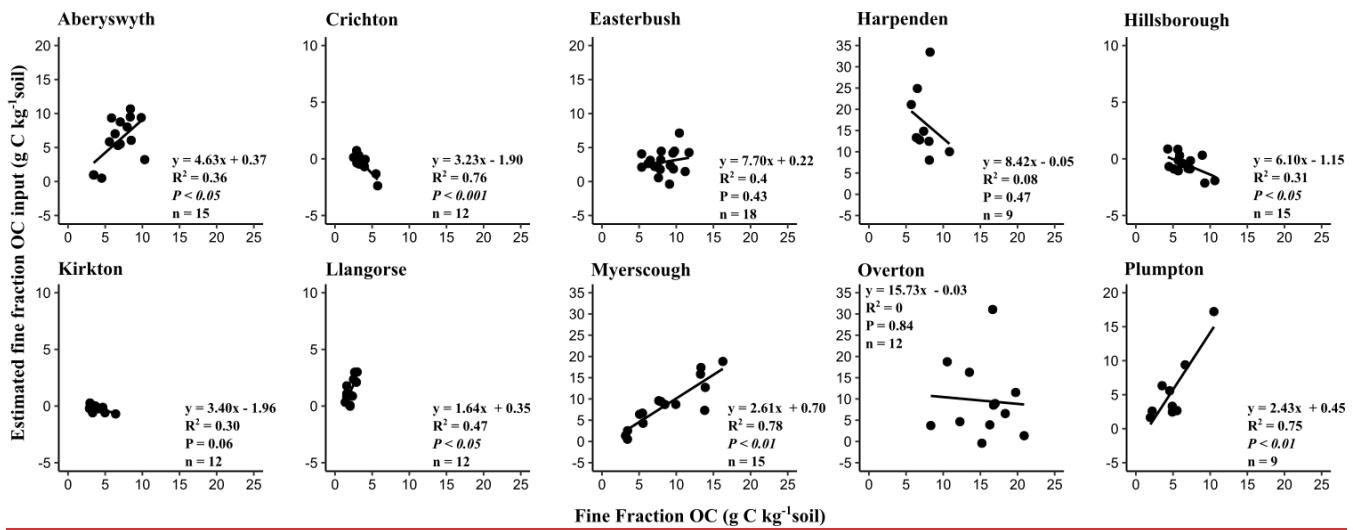


Figure A1. Estimated fine fraction OC input (g C kg⁻¹ soil) compared to measured fine fraction OC (g C kg⁻¹ soil) in each of the sites studied. The estimated OC input was predicted using quantile regression at the 90th percentile.