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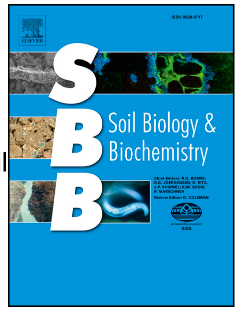

Quantifying the relationships between soil fraction mass, fraction carbon, and total soil carbon to assess mechanisms of physical protection

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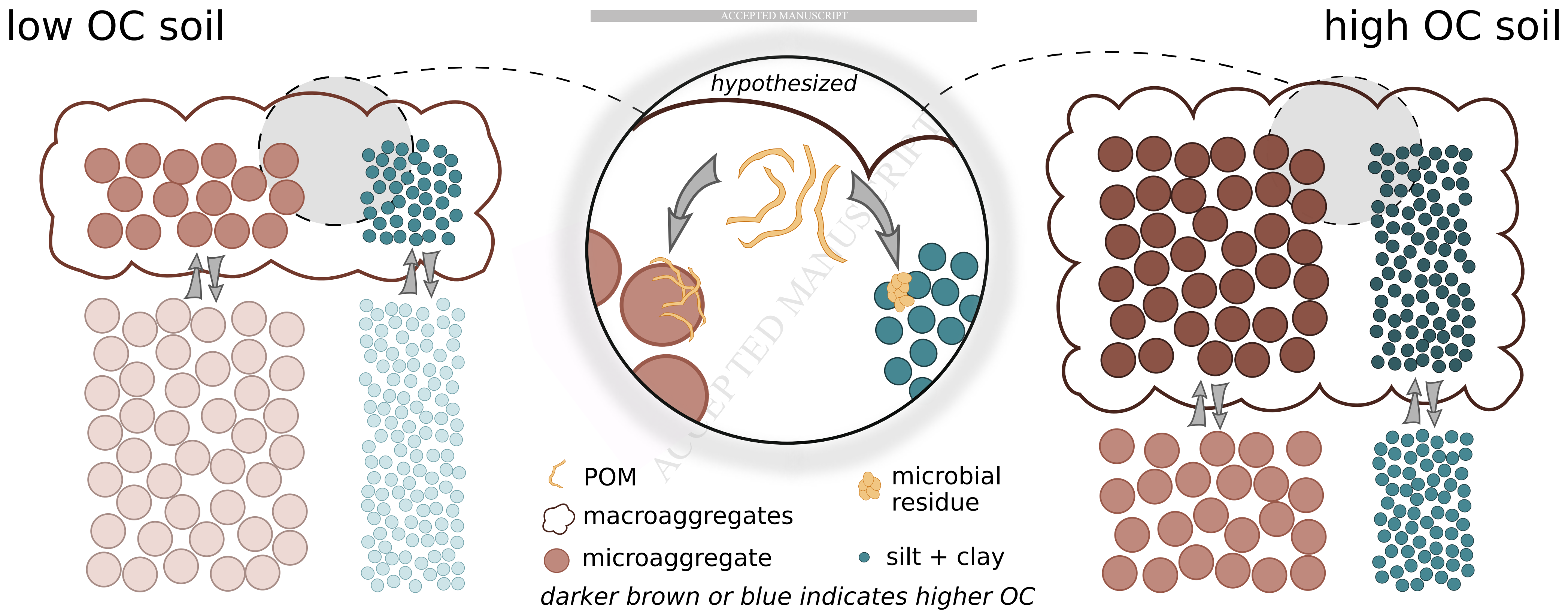
low OC soil
high OC soil
hypothesized
POM
macroaggregates
microaggregate
microbial residue
silt + clay
darker brown or blue indicates higher OC

Title

Quantifying the relationships between soil fraction mass, fraction carbon, and total soil carbon to assess mechanisms of physical protection

Authors

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Abstract

Relationships between soil fractions (their mass or carbon (C)) and soil organic carbon (SOC) have been used to develop central ideas in SOC research. However, few attempts have been made to quantify the relationship between SOC and all soil fractions, despite the potential of such an effort to address SOC stabilization processes. We identified 41 published studies that used diverse management techniques to cause a change in SOC concentration and disrupted soil into macroaggregates (> 250 μm), free microaggregates (53-250 μm) and free silt + clay (< 53 μm), subsequently disrupting macroaggregates into constituent fractions (coarse particulate organic matter [cPOM] > 250 μm, occluded microaggregates, and occluded silt + clay). We used linear hierarchical models to quantify relationships between mass, C concentration and total C of fractions and SOC. Soil mass redistribution toward macroaggregates was associated with SOC accumulation, however total microaggregate mass (free + occluded) did not increase with macroaggregate mass, as would be expected given *de novo* microaggregate formation within macroaggregates. Instead, high SOC soils exhibited a greater percent of total microaggregates occluded in macroaggregates. Occlusion in macroaggregates was also associated with increased C concentrations of microaggregates (35% higher, SE = 3.2) and silt + clay (30% higher, SE = 3.9) relative to their free counterparts. Taken together, these relationships suggest reduced macroaggregate turnover promotes SOC accumulation *via* the stabilization of C into occluded fractions. Rates of SOC increase with silt + clay C concentrations failed to increase with mean site-level SOC concentration, indicating of the studied soils (median SOC concentration = 14 g kg^{-1}; max 68), SOC accumulation appears unlikely to be limited by C storage capacity in the silt + clay fraction. For each unit SOC gain, macroaggregates accounted for 83% (95% CI = 74, 91),

and occluded microaggregates for 43% (95% CI = 33, 52), consistent relationships that have potential to be used as benchmarks for fraction-based SOC models.

1. Introduction

Soil organic carbon (SOC) represents a pool of C larger than terrestrial biomass and atmospheric C combined (Janzen, 2015). Mechanisms of SOC formation and persistence have long been a challenge to untangle, with physical protection, such as within soil aggregates, acknowledged as a primary mechanism in operation (Lehmann and Kleber, 2015; Schmidt et al., 2011). An improved understanding of physical protection mechanisms is needed to constrain the potential of SOC to mitigate climate change (Minasny et al., 2017) and to provide ameliorated soil health (Harden et al., 2018).

A key step in studying physical mechanisms of SOC protection is the identification of measurable soil fractions which can be used to make inferences about processes governing the formation and persistence of SOC (Sohi et al., 2001; Stewart et al., 2008). Many soil physical fractionation methods have been used to disrupt soil into such putative size or density fractions (Beare et al., 1994; Cambardella and Elliott, 1992; Elliott, 1986; Gupta and Germida, 1988; Kemper and Rosenau, 1986; Six et al., 2000a; Sohi et al., 2001). Usually, these studies rely on differences in soil management to create variability in total SOC and soil fractions so that relationships between them can be assessed (Beare et al., 1994; Cambardella and Elliott, 1992; Elliott, 1986; Gupta and Germida, 1988; Jastrow et al., 1996; Six et al., 2000a). These and similar investigations show 1) soil fractions differ in their stability to subsequent disruption, leading to the general conclusion that smaller soil fractions persist longer and therefore offer more stable protection to associated organic C (Jastrow et al., 1996) and 2) soils with a greater mass in larger fractions (usually > 250 μm) are associated with greater SOC levels (Beare et al., 1994; Elliott, 1986; Gupta and Germida, 1988), leading to a larger mean weight diameter. However, to our knowledge, few attempts have been made to quantify the relationship between SOC and all soil fractions, despite the potential of such an effort to address SOC stabilization processes (Harden et al., 2018). To test for relationships between SOC and soil fractions, it is essential that a quantitative literature review target a common soil fractionation method, thereby minimizing the variability arising from disparate methods.

The soil fractionation method of Six et al. (2000a) disrupts macroaggregates (>250 μm) into fractions occluded within them: coarse particulate organic matter (cPOM), occluded microaggregates (53-250 μm), and occluded silt + clay (<53 μm). Often used as an extension of the simpler one-step soil fractionation of Elliott (1986), it provides additional information by isolating intra-macroaggregate fractions. Although other techniques provide even more detailed information on soil fractions (Gupta and Germida, 1988; Sohi et al., 2001), Six et al. (2000a)'s method has been more widely applied (860 citations on Web of Science as of September 2017). Studies using this fractionation method typically derive soil fraction mass and fraction C concentration, which can then be multiplied to determine total fraction C.

Six & Paustian (2014) reviewed seven studies that used the Six et al. (2000a) method and found that most SOC accumulation occurred in occluded microaggregates. This finding suggested greater significance of the conclusion of Six et al. (2000a), who compared tilled and no-till soils and observed increases in the proportion of macroaggregate mass composed of occluded microaggregates under no-till. Based on this observation, Six et al. (2000a) suggested reduced rates of macroaggregate turnover under no-till enabled occluded microaggregate formation and acted as a mechanism for SOC accumulation.

Based on the wide-spread use of the Six et al. (2000a) method, there is an opportunity to further explore the relationships between SOC levels and all soil fractions through a quantitative review across many sites. For instance, including silt + clay and cPOM fractions in a review has potential to inform because their carbon dynamics are central to C saturation theory (Hassink, 1997; Six et al., 2002). This theory holds that the C adsorption capacity of silt + clay is limited, and that once this fraction is C saturated, C accrual must occur as free POM (Gulde et al., 2008; Stewart et al., 2008) or macroaggregate-occluded POM (Brown et al., 2014). Whether C saturation is widespread in soils has so far received inconclusive support (Stewart et al., 2008, 2007). It is also possible that other soil dynamics are sensitive to SOC concentration, such as rates of aggregate mass redistribution, however this possibility has received little theoretical attention to our knowledge. Parsing the relationship between changes in each fraction mass, fraction C concentration, and total fraction C could address several additional unknowns: it is unknown if preferential increases in total fraction C occur due to a redistribution of soil mass into that fraction, due to higher rates of increase in the C concentration of that fraction, or some combination of these two processes. Evaluating microaggregate dynamics with SOC accumulation can also help to test the proposition that macroaggregate turnover enables SOC accumulation via microaggregate formation (Six et al., 2000a) across multiple sites. Six et al. (2000a) found increases in mass of occluded microaggregate as a proportion of macroaggregates was associated with increases in SOC concentration and linked this to decreases in macroaggregate turnover. Investigating these relationships across multiple studies requires careful selection of statistical techniques.

Quantitative reviews in soil ecology have relied on a suite of increasingly precise statistical techniques (Gurevitch et al., 2018). From early use of vote-counting, limited by bias and imprecision (Gurevitch and Hedges, 1999), formal techniques in meta-analysis rely on the pair-wise comparison of treatment and control observations within the same study to generate standardized effect sizes (Gurevitch et al., 2018; Koricheva and Gurevitch, 2014, e.g., King and Blesh, 2018; Tonitto et al., 2006). Effect sizes do not quantify variables in absolute units (e.g., g SOC kg soil^{-1}), but absolute changes can be estimated in quantitative reviews through the use of regression (Booth et al., 2005; West and Post, 2002). Linear hierarchical models provide a particular advantage for the nested datasets often generated by quantitative reviews (i.e., multiple treatments within sites) because they can synthesize all within-site slopes into global slopes, which are then not confounded by drivers of between-site variation (Woltman et al., 2012). Linear hierarchical models can also be adapted to answer specific questions in soil ecology, such as whether C saturation occurs between (but not within) sites, by extracting lower-level slopes and testing whether they change with mean site-level SOC concentration.

To examine the relationship between SOC, fraction mass, fraction C concentration, and total fraction C, we systematically reviewed studies that used the macroaggregate fractionation method of Six et al. (2000a). We addressed the following specific questions: 1) When all soil fractions are considered, do occluded microaggregates accumulate C preferentially as SOC increases? 2) Is preferential C accumulation in any soil fraction caused by changes in that fraction's C concentration, mass, or both? 3) Do fraction and SOC relationships support *de novo* formation of microaggregates within macroaggregates? 4) Are rates of change in soil fraction characteristics (total C, C concentration, and mass) with SOC concentration moderated by mean site-level SOC concentration? Given the hypothesized role of macroaggregation in mechanisms of SOC accumulation, we also asked 5) Does occlusion in macroaggregates influence C concentrations of microaggregates and silt + clay particles? Environmental variables (MAT, MAP, clay, elevation, pH, soil order) have also received much attention as potential between-site drivers of macroaggregation and SOC (Burke et al., 1989; Denef et al., 2007; Doetterl et al., 2015; Percival et al., 2000; Rasmussen et al., 2018), hence, we tested for their power in explaining between-site variability in SOC concentration and macroaggregate mass.

2. Materials & Methods

2.1 Database search

In September 2017, we searched Web of Science Core Collection for all papers citing Six et al. (2000a) (approximately 830 studies). Criteria for inclusion in the review were that studies 1) used the method of Elliott (1986) to sieve field-moist soil to 8 mm, allow to air-dry, then wet-sieve into macroaggregates, free microaggregates, and free silt and clay; 2) used the method of Six et al. (2000a) to isolate occluded fractions (coarse POM, microaggregates, and silt + clay), and 3) reported either a mass, C concentration, or total C value for an occluded fraction. We updated the search in January 2018. We refer readers to Figure 1 for sieving method and size classifications common to studies reported and to Six et al. 2000a for more detailed descriptions of the method.

Studies found with these criteria reported SOC (concentration or total on a mass per area basis) and soil fractions as a response to a variety of management interventions, in some cases at multiple depths. In some studies, macroaggregates were reported separately by large (>2 mm) and small (250 μm – 2 mm) macroaggregates. For each treatment and depth increment, we recorded data for as many of the following seven soil fractions as were reported: large macroaggregates, small macroaggregates, free microaggregates, free silt + clay, occluded cPOM, occluded microaggregates, and occluded silt + clay. Hereafter, we refer to occluded cPOM as simply cPOM. Data collected on soil fractions included: mass (g fraction 100 g soil^{-1}, or, for occluded fractions, g fraction 100 g macroaggregate^{-1}), C concentrations (g C kg fraction^{-1}, or for occluded fractions, per kg macroaggregate), and percent total SOC in fraction or percent macroaggregate C in occluded fraction. We also recorded total soil C concentration (g kg soil^{-1}), percent sand, silt and clay (if reported separately by treatment), upper and lower depth of soil sampling, and land cover (agriculture, forest, or grassland). We recorded management practices used as treatments and USDA soil orders, and we translated soil orders reported in the FAO classification to USDA soil orders where possible.

For each site, we noted elevation (m), mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm), and percent sand, silt, and clay. Where necessary, we used Data Thief (Tummers, 2006) to extract data from figures. Where elevation was not reported, we estimated it based on latitude and longitude (https://www.geoplaner.com/).

2.2 Calculations

The diversity of units used to report soil fraction C and mass resulted in a preliminary database that needed to be standardized. We combined large and small macroaggregates (hereafter ‘macroaggregates’), reducing the total number of soil fractions analyzed to 6. Where large and small macroaggregates were reported separately in terms of g macroaggregate per 100 g soil, g macroaggregate C per kg soil, or percent total soil C in macroaggregates, we calculated total macroaggregate mass or C as the sum of large and small macroaggregate values. Where the concentration of macroaggregate C, occluded microaggregate C, or occluded silt + clay C was reported separately for large and small macroaggregates (LM and SM, respectively), we calculated total macroaggregate or macroaggregate-occluded C as a mass-weighted average:

$$g\ LM\ C\ kg\ soil^{-1} * \frac{g\ LM\ g\ soil^{-1}}{g\ LM\ + SM\ g\ soil^{-1}} + g\ SM\ C\ kg\ soil^{-1} * \frac{g\ SM\ g\ soil^{-1}}{g\ LM\ +\ SM\ g\ soil^{-1}}$$

When total fraction C was not reported, we multiplied g fraction kg soil^{-1} by g fraction C g fraction^{-1}, or alternatively, we multiplied g fraction C g soil C^{-1} by g soil C kg soil^{-1} to obtain g fraction C kg soil^{-1}. To express fraction C concentration where it had not been reported, we multiplied g fraction C kg soil $^{-1}$ by kg soil kg fraction $^{-1}$ to obtain g C kg fraction^{-1}. We calculated aggregate mean weight diameter (De Gryze et al., 2006), using separate diameters for large and small macroaggregates if they were reported, and otherwise we used a single diameter for total macroaggregates.

At 8 sites, total SOC concentration was not reported. Therefore, we calculated total SOC concentration as the sum of g C fraction kg soil^{-1} for large and small macroaggregates, free microaggregates, and free silt + clay. If necessary, when both mass distribution and C concentration per fraction were reported, we calculated g C fraction kg soil^{-1} as above and then summed fraction C to arrive at total SOC. In these cases, only C concentrations for non-sand-corrected aggregate concentrations were used. This measure avoided overestimating total SOC concentration. In some cases, fraction C or total SOC numbers were reported on a mass per area basis. We used bulk density numbers specific to treatment and depth increments to convert C per area measurements to a concentration basis, contacting study authors where necessary for bulk density values.

2.3 Statistics

To test for relationships between four soil fractions characteristics (fraction mass, mean weight diameter, fraction C concentration, and total fraction C) and SOC concentration, we constructed linear mixed-effect hierarchical models, using the R package lme4 (Bates et al., 2015). Hierarchical models were necessary given the nested structure of data (treatments within sites, e.g., Senior et al. (2018)), and enabled estimation of within-site slopes that are then combined into global slopes, so any effect of environmental variables (clay, MAP, MAT, etc.) on mean site-level SOC or fraction characteristics does not appear in the global slope. In choosing dependent and independent variables for the hierarchical models, we combined pragmatic and mechanistic approaches: pragmatically, in order to estimate the percent of total fraction C apportioned to each fraction per unit SOC concentration gained, we used total fraction C as the dependent variable and SOC concentration as the independent variable, because this model creates slopes that correspond to the needed formulation (Δ total fraction C / Δ SOC). In recognition of physical protection mechanisms governing SOC accumulation, we used SOC concentration as the response variable for other models (fraction mass, fraction C concentration, mean weight diameter).

For the random effect for all models, we used unique site and depth identifiers assigned after grouping soil observations from within the same site and depth. This grouping avoided confounding an effect of treatment with an effect of depth, however, for simplicity, we hereafter use the term *site* to denote a single depth increment within site. We set models to generate separate slopes and intercepts for each site, and we consider these slopes to be the management-induced effects on aggregation and SOC concentration. Model output also included global slopes and intercepts for the model. For global slopes, we calculated 95% confidence intervals (hereafter "CI"). As our models included a random effect for site, it was necessary to calculate R^2 values considering the model both with the random effect (conditional R^2, or R^2C), and without (marginal R^2, R^2M), which were extracted from linear hierarchical models using the MuMIn package (Nakagawa and Schielzeth, 2013). Although not all data displayed homogeneity of variance, we consider the model reasonably robust to these effects (Zuur et al., 2009) and we avoided transforming variables because the production of ecologically meaningful slopes was a leading objective of the review. Specifically, however, residual distribution in linear hierarchical models formed slightly heteroscedastic, unbiased distributions around predicted values, with higher SOC concentrations corresponding to slightly larger residuals and sparser data. We acknowledge that uncertainty around the rate of change between SOC and soil fraction characteristics is greater at higher ranges of SOC concentration (> 40 g SOC / kg soil) than at lower SOC concentrations.

We were interested in exploring if mean site-level SOC concentration affected the rate of mass, C concentration, or total C change in soil fractions (described above and in SI Figure 2); that is, if the within-site slopes generated from linear hierarchical models changed as a function of SOC concentration for each of the six soil fractions. To check trends of within-site slopes, we used linear regression between slopes (dependent variable) and mean site-level SOC concentrations (independent variable).

To test for the effect of environmental variables (MAT, MAP, elevation, clay and pH) on SOC and macroaggregate mass, we used linear regression, with SOC g kg soil^{-1} or macroaggregate mass as the dependent variable and the environmental variable as the independent variable, taking only the upper-most reported depths from each site. Linear regression (without a hierarchical component) was necessary because environmental variables had only one level for each site (with few exceptions, e.g., Brown et al., 2014). For these environmental analyses only, SOC concentrations were log-transformed where necessary for residuals to meet assumptions of normality, and log-transformations are indicated where regressions are reported. As macroaggregate mass displayed a boundary-line relationship with MAP, we performed an additional boundary-line regression to identify the minimum rate of macroaggregate increase with MAP. To perform boundary-line analysis, we identified upper and lower bounds of reported MAP (excluding outlier MAP > 3,000 mm), divided data into ten equal increments along the MAP gradient, and then took the lowest 10% of reported macroaggregate values in each increment; these data were then used in linear regression (Feng et al., 2013). To test for the effect of soil order on SOC concentrations and macroaggregate mass, we performed one-way ANOVAs, with soil order as a categorical predictor variable. As all treatments (representative of management) within sites had the same values for these environmental variables (with some exceptions for clay), we consider these tests to represent the effect of environment on aggregation and SOC concentration. All analyses were carried out in R v. 3.3.3 (Core Team, 2017).

2.4 Caveats and limitations of the dataset

Despite overall consistency of fractionation methods used in the study, not all studies used the same procedures for sand correction (Elliott et al., 1991) and correction for light fraction (Six et al., 1998). Out of all sites, 43% performed sand corrections on soil fractions, while 56% did not, thus, the cPOM fraction should be considered 'cPOM *or* cPOM + sand'. Corrections for free light fraction were carried out at 10% of sites, while 29% performed light fraction correction on a subset of aggregate fractions (usually occluded microaggregates) and 60% reported no light fraction corrections at all. We acknowledge that these differences in sand and light fraction correction likely contributed to variability in the dataset. Not all sites were accompanied by reports of soil texture, soil order, or other environmental variables. We note that some soil fractions were reported more consistently than others, and that the CI of slopes of SOC concentration and soil fraction values reflect both the variability of the reported relationships as well as the frequency of reporting. We acknowledge that mean site-level SOC concentration is influenced by depth of sampling, which was variable throughout the top 40 cm of soil (SI Table 1). We included all available observations to avoid loss of data and our selection of random effects in hierarchical models ensured that changes in SOC and fraction characteristics were not confounded by change in soil depth.

3. Results

3.1 Database synthesized from literature

Our search resulted in a database (SI Table 1) of 41 studies reporting from 38 sites (Ayuke et al., 2011; Bhattacharyya et al., 2012; Bossuyt et al., 2004; Brown et al., 2014; Carrington et al., 2012; Chung et al., 2010, 2008; Das et al., 2014; Del Galdo et al., 2003; Denef et al., 2007; Fahey et al., 2013; Fonte et al., 2014, 2010a, 2010b, 2009b, 2009a, 2007; Fonte and Six, 2010; Fultz et al., 2014, 2013b, 2013a, Gentile et al., 2011, 2010; Gulde et al., 2008; Hoosbeek et al., 2006; Huang et al., 2010; Jiang et al., 2017; Knowles et al., 2016; Kumari et al., 2011; Liang et al., 2014; Lichter et al., 2008; Mayzelle et al., 2014; Nicolás et al., 2014; Oorts et al., 2007; Scott et al., 2017; Sheehy et al., 2015; Simpson et al., 2004; Singh et al., 2015; Yavitt et al., 2015; Zhao et al., 2018; Zotarelli et al., 2005). Studies were located on five continents (Australia and Antarctica not represented), with reports from North America (39% of studies) and Asia (18% of studies) most common. Studies that reported soil orders included reports from Alfisols, Entisols, Inceptisols, Mollisols, Oxisols, and Ultisols (SI Table 3), with 8 studies not reporting a USDA soil order. No studies were known to report from Gelisols, Andisols, Vertisols, Spodosols, or Histosols, but since 8 studies did not report USDA soil order it is possible some of these orders were included, however we acknowledge that patterns of SOC accumulation discussed are not expected to extend to these soil orders. Additional information on the range of environmental variables across studies can be found in the Supplementary Information. Studies reported results from treatments applied to experimental sites in agriculture, grassland, forest, or a mixture of these, with the majority (76%) of studies reporting agricultural sites. Studies used a range of treatments, including: tillage, species identity or diversity (usually through crop rotation), inorganic nitrogen (N) fertilizer, quality of organic amendment, quantity of organic amendment, harvest practices, earthworm abundance or species, free air carbon enrichment, and restoration (from agriculture to grassland). Sites had a minimum of 2 different treatments, with a mean of 4.3, resulting in a total of 199 treatments in the database. As some studies reported soil at multiple depths (11 out of 38 sites), which we retained as separate observations, the database contained a total of 253 unique site *x* treatment *x* depth soil observations. Maximum depth of sampling was 40 cm, with the most common depth increment being 0-15 cm (14 out of 38 sites).

Figure 1.

3.2 Do occluded microaggregates accumulate C preferentially as SOC increases?

All data for the relationship between SOC concentration and total fraction C are shown in Figure 2 and regression coefficients for the global slopes generated from linear hierarchical models are presented in Table 1 (section *Total Fraction C / SOC Concentration)*. Given a perfect accounting of total C in soil fractions (and assuming no correction for free light fraction), the global slopes for the relationship between total fraction C accumulation for macroaggregates, free microaggregates, and free silt + clay would sum to 1. Global slopes estimated from the review were remarkably close to this target: for each unit SOC increase, 0.89 units of fraction C were accounted for on average. Hereafter, we translate global slopes reported in Table 1 (section *Total Fraction C / SOC Concentration)* to percentages by multiplying by 100; these percentages represent the percent of SOC accumulation that occurs in each fraction.

Among all six fractions, macroaggregates accumulated total C at both the highest rate and with the greatest consistency (83%, CI = 74, 91%, Table 1, Figure 2 *Macroaggregate*). All fractions except macroaggregates were non-overlapping (i.e., macroaggregates contained occluded fractions that were reported both as part of macroaggregates and individually). Within the set of non-overlapping fractions, occluded microaggregates accumulated total C at the highest rate (43%, CI = 33 - 52%; Table 1, Figure 2 *Occluded microaggregate*). Among all three macroaggregate-occluded fractions, occluded microaggregates preferentially accumulated C as SOC increased, gaining more C than both other fractions combined (cPOM and occluded silt + clay, Table 1). Free microaggregates were responsible for little C gain and the free silt + clay fraction often lost C as SOC concentration increased (Table 1, Figure 2).

3.3 What soil fraction dynamics explain preferential C accumulation?

All data for the relationship between fraction C concentration and SOC concentration are shown in Figure 3 and regression coefficients for the global slopes generated from linear hierarchical models are presented in Table 1 (section *SOC Concentration / Fraction C Concentration).* Among all soil fractions, increases in C concentrations were associated with similar increases in

SOC concentrations, with the exception of cPOM (Figure 3, Table 1): a 1 g increase in fraction C concentration (except in cPOM) was associated with a ~0.8 g increase in g SOC kg soil $^{-1}$, and CI intervals for global slopes overlapped for the different fractions (Table 1). For cPOM, however, a 1 g increase in C concentration was associated with a 0.09 g increase in SOC kg soil^{-1} (CI = 0.05, 0.17, Table 1). As rates of C concentration increase were similar across fractions (except cPOM), it follows that changes in fraction mass (i.e. redistribution) explained the preferential increase in total fraction C with SOC concentration for a given soil fraction.

All data for the relationship between fraction mass and SOC concentration are shown in Figure 4 and regression coefficients for the global slopes generated from linear hierarchical models are presented in Table 1 (section *SOC Concentration / Fraction Mass)*. Soil organic carbon consistently increased with the redistribution of soil mass into macroaggregates, away from free microaggregates or free silt and clay (Figure 4, Table 1). Specifically, with each additional 1 g macroaggregate 100 g soil^{-1}, there was an increase of 0.27 g SOC kg soil^{-1} (CI = 0.18, 0.35, Table 1). Among occluded fractions on a whole soil basis, increases in both occluded microaggregates and occluded silt + clay were associated with increases in SOC concentration (Table 1, Figure 4), with global slope estimates between 0.23 g SOC kg soil^{-1} for each 1 g occluded microaggregate 100 g soil^{-1} and 0.46 g SOC kg soil^{-1} for each additional 1 g occluded silt + clay (CI for these slopes did not differ). Coarse POM mass had the most variable relationship to SOC concentrations of all fractions reported, within increases in cPOM sometimes associated with an increase and sometimes with a decrease in SOC concentrations (CI = -0.56, 1.42, Table 1, Figure 4 *cPOM*).

Increases in mean weight diameter of aggregates were associated with higher SOC concentrations (8.8 g SOC kg soil $^{-1}$ / mm, CI = 5.3, 12.3; SI Figure 3). Rates of SOC accumulation with changes in aggregate mass appeared to be moderated by mean site-level SOC concentrations, a relationship quantified below (section 3.5, SI Figure 2, and Table 3).

Table 1. Global regression coefficients generated from linear hierarchical models, quantifying the relationship between SOC concentration and total fraction C, fraction C concentration, and fraction mass. Marginal R^2 (R^2M) values represent the explanatory power of the relationship between SOC concentration and fraction characteristic without a random effect for site. All conditional R^2 values (those incorporating random effect of site) were above 0.79. Confidence intervals (CI) represent 95% confidence intervals of the global slope.

Fraction	**Sites**	**Obs.**	**Intercept**	**Slope**	**CI**	**R^2M**
	Total Fraction C / SOC Concentration[*]					
Macroaggregate	51	219	-3.13	0.83	0.74, 0.91	0.85
Free microaggregate	52	221	2.88	0.08	0.03, 0.12	0.12
Free silt + clay	51	217	1.88	-0.02	-0.04, 0.01	0.03
cPOM	35	163	-0.84	0.14	0.05, 0.23	0.21
Occluded microaggregate	37	167	-1.16	0.43	0.33, 0.52	0.63
Occluded silt + clay	34	157	0.97	0.16	0.09, 0.23	0.24
	SOC Concentration / Fraction C Concentration[Φ]					
Macroaggregate	50	216	4.28	0.72	*0.57, 0.86*	0.57
Free microaggregate	50	216	5	0.93	0.73, 1.13	0.65
Free silt + clay	50	214	6.42	0.83	0.54, 1.12	0.33
cPOM	28	118	17.54	0.11	0.05, 0.17	0.22
Occluded microaggregate	29	124	5.96	0.68	0.42, 0.93	0.53
Occluded silt + clay	28	121	3.08	0.86	0.57, 1.15	0.59
	SOC Concentration / Fraction Mass[⊥]					
Macroaggregate	52	216	3.79	0.27	0.18, 0.35	0.25
Free microaggregate	53	218	27.71	-0.28	-0.38, -0.18	0.14

Free silt + clay	52	211	25.66	-0.42	-0.53, -0.3	0.14
cPOM	24	97	18.57	0.43	-0.56, 1.42	0.04
Occluded microaggregate	30	119	14.01	0.23	0.14, 0.32	0.08
Occluded silt + clay	24	97	15.33	0.46	0.12, 0.8	0.05

[*] Unit for the slope is (g fraction C / kg soil) / (g SOC / kg soil), or (fraction C / SOC); intercepts in g fraction C / kg soil

[Φ] Unit for the slope is (g SOC / kg soil) / (g fraction C / kg fraction); intercepts in g SOC / kg soil

[⊥] Unit for the slopes is (g SOC / kg soil) / (g fraction / 100 g soil); intercepts in g SOC / kg soil

Figure 2.

Figure 3.

Figure 4.

Figure 5.

3.4 Do fraction and SOC relationships support de novo *formation of microaggregates within macroaggregates?*

Total microaggregate mass did not increase with macroaggregate mass (Figure 5.A, slope = 0.06 g total micro. 100 g soil^{-1} / g macro. 100 g soil^{-1}, CI = -0.11, 0.24, R^2M = 0.01), and furthermore, total microaggregate mass was associated with relatively small changes in SOC concentration (Figure 5.B, slope = 0.08 g SOC kg soil^{-1} / g total micro. 100 g soil^{-1}, CI = -0.04, 0.20, R^2M = 0.01). Instead, significant increases in SOC concentration were caused by increases in the percent of total microaggregates occluded in macroaggregates (Figure 5.C, slope = 0.21 g SOC kg soil^{-1} / g occ. micro. 100 g total micro^{-1}, CI = 0.14, 0.28, R^2M = 0.14). Increases in the proportion of total (free + occluded) silt + clay occluded in macroaggregates were also associated with increases in SOC concentration (SI Figure 4).

Although the proportion of total microaggregates within macroaggregates explained SOC concentrations, we did not detect a consistent trend between the percent macroaggregate mass or C in occluded microaggregates or cPOM and SOC concentration (Table 2, SI Figure 5). Among all three occluded fractions, a consistent (decreasing) trend of SOC concentration with percentage of macroaggregate mass or C in occluded form was only observed for silt + clay (Table 2).

Table 2. Global regression coefficients generated from linear hierarchical models, with SOC concentration as a dependent variable and occluded fractions as a percent macroaggregate mass or C as independent variables. Marginal R^2 (R^2M) values represent the explanatory power of aggregate characteristics on SOC concentrations without random effect for site. All conditional R^2 values (those incorporating random effect of site) were above 0.89. Confidence intervals (CI) represent 95% confidence intervals of the global slope.

	Sites	Obs.	Intercept*	Slope$^{\varphi}$	CI	R^2M
Percent Mass in Macroaggregate						
cPOM	23	95	21.60	-0.14	-0.50, 0.21	0.02
Occluded microaggregate	29	117	14.22	0.09	-0.08, 0.27	0.02
Occluded silt + clay	23	95	32.82	-0.37	-0.61, -0.13	0.08
Percent C in Macroaggregate						
cPOM	34	161	18.91	0.02	-0.3, 0.33	0
Occluded microaggregate	36	165	17.07	0.06	-0.12, 0.24	0.01

Occluded silt + clay	34	157	29.91	-0.32	-0.49, -0.16	0.08

* Units are in g SOC / kg soil

φ Units are in g SOC kg soil $^{-1}$ / fraction unit, where fraction units are defined in the far left column: e.g., Percent Mass in Macroaggregate, cPOM is (g cPOM / g macroaggregate) * 100.

3.5 Are rates of change in soil fraction characteristics with SOC concentration moderated by mean site-level SOC?

We used mean site-level SOC concentration as a predictor variable in a simple linear regression to test for a trend in slopes (response variable) generated by the linear hierarchical models (SI Figure 2, Table 3). The relationship between SOC concentration and total fraction C show little relationship to mean site-level SOC concentration, as indicated by low R^2 values (-0.02 – 0.12, Table 3, *Fraction Total C*). Similarly, the relationship between SOC concentration and fraction C concentration showed little relationship to mean site-level SOC concentration (R^2 = 0.02 – 0.30, Table 3**,** *Fraction C Concentration*). However, mean site-level SOC concentration did moderate fraction mass, as sites with higher average SOC concentrations displayed a greater response in SOC for increases in macroaggregate mass, as shown by a positive slope in Table 3, *Fraction Mass*. A similar significant moderating response of mean site-level SOC on fraction mass redistribution was observed for all other fractions except cPOM.

Table 3. Linear regression coefficients generated by using mean site-level SOC concentration as an independent variable and site-level slopes of the hierarchical linear regression between SOC concentration and total fraction C, fraction C concentration, and fraction mass as dependent variables (data shown by Figures 2 - 4 as indicated below). All SOC concentrations in g SOC / kg soil.

	Sites	P-value	Slope	R^2
Fraction Total C (Fig. 2)	*Slope = (Fraction Total C / SOC) / mean site-level SOC*			
Macroaggregate	51	0.0570	0.002	0.05
Free microaggregate	52	0.9238	0	-0.02
Free silt + clay	51	0.8663	0	-0.02
cPOM	35	0.0230	0.004	0.12
Occluded microaggregate	37	0.4814	-0.001	-0.01
Occluded silt + clay	34	0.6003	-0.001	-0.02
Fraction C Concentration (Fig. 3)	*Slope = (SOC / Fraction C Concentration) / mean site-level SOC*			
Macroaggregate	50	0.0075	-0.004	0.03
Free microaggregate	50	< 0.00001	-0.013	0.28
Free silt + clay	50	< 0.00001	-0.01	0.07
cPOM	28	0.0029	0.001	0.07
Occluded microaggregate	29	< 0.00001	-0.013	0.30
Occluded silt + clay	28	0.0941	-0.004	0.02
Fraction Mass (Fig. 4)	*Slope = (SOC / Fraction Mass) / mean site-level SOC*			
Macroaggregate	52	< 0.00001	0.013	0.68
Free microaggregate	53	< 0.00001	-0.012	0.72
Free silt + clay	52	< 0.00001	-0.021	0.80
cPOM	24	0.1048	0.015	0.02
Occluded microaggregate	30	< 0.00001	-0.003	0.91
Occluded silt + clay	24	< 0.00001	0.017	0.97

3.6 Does occlusion in macroaggregates influence fraction C concentration?

In the same soil, occluded microaggregates and occluded silt + clay showed higher C concentrations than their free counterparts (Figure 6), as observations fell above the 1:1 line between occluded and free fractions. Occluded silt + clay was on average 30% (SE = 3.9 g C / kg silt + clay) higher than free silt + clay. Occluded microaggregates were on average 35% (SE = 3.2 g C / kg microaggregate) higher in C kg^{-1} than free microaggregates.

Figure 6.

3.7 Environmental properties as predictors of SOC concentration and macroaggregate mass

Among four environmental properties tested (MAT, MAP, clay, elevation and pH), several were statistically significant, but none explained more than 33% of the variability in SOC concentration between sites (pH, SI Table 2). Macroaggregate mass was not well predicted by soil percent clay (R^2 = 0.03, SI Table 2), with MAP explaining the most variability (R^2 =0.16, SI Table 2). Minimum reported values for macroaggregate mass increased linearly with MAP, and in a boundary-line analysis (with only the lowest 10% of macroaggregate values for each decile of MAP) MAP was a strong predictor of macroaggregate mass (R^2 = 0.89, Figure 7). Soil organic carbon was lower in Inceptisols than in Entisols, Mollisols, or Oxisols, with Alfisols and Ultisols displaying intermediate SOC concentrations (SI Table 3; P = 0.00102). Macroaggregation differed among USDA soil orders: Oxisols exhibited greater macroaggregation than Alfisols, Ultisols, and Mollisols, while Inceptisols exhibited greater macroaggregation than Mollisols (SI Table 3; P < 0.0001; Tukey's HSD).

Figure 7

4. Discussion

While physical protection in soil fractions has been linked to SOC stabilization for decades (Elliott, 1986; Tisdall and Oades, 1982), and more recent work points to the prominence of occluded microaggregates in SOC accumulation (Six et al., 2000a; Six and Paustian, 2014), we provide the first quantitative review to our knowledge to link a comprehensive set of soil fractions, including all free and macroaggregate-occluded fractions, to SOC concentration.

Having addressed our specific research questions in the results, we now highlight how these findings relate to ongoing discussions in SOC research, specifically: *4.1*) The role of environmental properties in predicting macroaggregate mass, *4.2*) Accumulation of soil organic carbon promoted by reduced macroaggregate turnover; *4.3*) Testing for carbon-saturating behavior of the silt + clay fraction; *4.4)* The SOC-dependent relationship between SOC and aggregate mass, and *4.5*) Implications of soil fraction relationships with SOC for models and indicators.

4.1 The role of environmental properties in predicting macroaggregate mass

The macroaggregate mass and SOC data (Figure 4 *Macroaggregate*) show that soils supported high levels of macroaggregate mass even in the lowest quartile of SOC concentrations (below 10 g / kg soil). This points to the role of inorganic binding agents in stabilizing macroaggregates (Bronick and Lal, 2005). A role for inorganic binding agents is also highlighted in the relationship between MAP and macroaggregate mass (Figure 7). Minimum macroaggregate mass increased linearly with MAP, potentially through the indirect effect of MAP in promoting the formation of amorphous Fe and Al oxides, previously shown to increase macroaggregate mass (Duiker et al., 2003; Wei et al., 2016). We found that clay content had little explanatory power over macroaggregate mass (R^2 = 0.03, SI Table 2), further pointing to the activity of clay, rather than total clay, as a determinant of macroaggregate mass (*sensu* Doetterl et al., 2018).

4.2 Accumulation of soil organic carbon promoted by reduced macroaggregate turnover

A key idea from Six et al., (2000a) held that slower macroaggregate turnover promotes SOC accrual by enabling the formation of occluded microaggregates. Given that reduced turnover is likely to induce increases in macroaggregate mass, our results (Figure 4 *Macroaggregate*) support the idea of reduced macroaggregate turnover promoting SOC accumulation. However, this reduced macroaggregate turnover does not appear to cause SOC accumulation *via* microaggregate formation according to the review data. If this were the prevalent mechanism, then more total (free + occluded) microaggregates should be associated with macroaggregate mass increase and increases in the total microaggregate mass should be associated with SOC accumulation. But, we find that across sites, reduced macroaggregate mass (as proxy for turnover) did not increase the mass of total microaggregates (Figure 5.A). Furthermore, increases in total microaggregate mass were associated with only small gains in SOC (Figure 5.B). These observations are consistent with the concept that soil mass in microaggregates is controlled by diverse forces of which macroaggregate turnover is not a primary agent (Totsche et al., 2017).

Rather than SOC accumulation *via* total microaggregate formation, we find SOC accumulation *via* larger C concentrations of macroaggregate-occluded fractions (microaggregates and silt + clay) relative to their free counterparts (Figure 6). Higher C concentrations of these macroaggregate-occluded fractions is consistent with delayed decomposition rates within the macroaggregate relative to outside of it (John et al., 2005; Sexstone et al., 1985), however, higher C concentrations may also be explained by enhanced rates of C accumulation in these macroaggregate-occluded fractions. Particulate organic matter, such as is derived from roots, constitutes an important source of C for soils (King and Blesh, 2018; Kong and Six, 2010; Rasse et al., 2005). Stabilization of POM occurs through its transfer to soil compartments with a slower turnover, such as through the production of microbial residues that adsorb to mineral surfaces (Kallenbach et al., 2016; Kögel-Knabner et al., 2008) or the occlusion of fine POM within microaggregates (John et al., 2005; Mccarthy et al., 2008). Macroaggregates support an abundance of microbial biomass relative to free microaggregates and silt + clay (Chen et al., 2014; Gupta and Germida, 1988), and the abundance of this microbial biomass and its proximity to microaggregate and silt + clay surfaces, we suggest, promotes stabilization of POM into these fractions.

The concept that macroaggregates provide an advantageous environment for the stabilization of POM is further supported by studies investigating the fate of specific C inputs to soil. Within the first month after POM addition, almost all intra-aggregate POM was found in macroaggregates, rather than stabilized in free microaggregates or absorbed to free silt and clay (Andruschkewitsch et al., 2014; Angers et al., 1997; Li et al., 2016; Sánchez-de León et al., 2014). Over the course of two years, recovery of ^{13}C-labeled POM increased in size classes of free microaggregate and free silt and clay C (Angers et al., 1997; Jin et al., 2018; Li et al., 2016), a timeline consistent with multiple cycles of macroaggregate formation and disruption, which are estimated at 5-90 days (De Gryze et al., 2006, 2005; Plante et al., 2002). Moreover, when density fractionation was performed to remove free POM, applied ^{13}C tracer recovery also increased in free microaggregates and on free silt + clay, showing ^{13}C had been incorporated onto these more stable forms (Angers et al., 1997; Li et al., 2016). Taken together, these studies and our results (Figure 6) suggest that when POM is incorporated into macroaggregates, its C is more efficiently stabilized *via* occlusion into microaggregates or *via* the production of microbial residues which are stabilized on silt + clay surfaces (Angst et al., 2017; Kirchmann et al., 2004; Plaza et al., 2013). A higher efficiency of C stabilization in macroaggregates would explain the positive relationship between percent of total microaggregates occluded in macroaggregates and SOC concentration (Figure 5.C).

Methodological differences between studies could have contributed to the observed increase in C concentration of the occluded microaggregate size class relative to free, as not all studies applied sand and light fraction corrections. A higher occluded microaggregate C concentration compared to free microaggregates would be inflated by more fine POM or lower concentration of sand inside the macroaggregate compared to outside. However, even when considering only studies that applied sand correction, occluded microaggregates were still 19% (SE = 4.8) higher on average in C concentration than free microaggregates. Our finding that the silt + clay C concentration is increased when this fraction is occluded (Figure 6) is less likely to be affected by methodological differences, as the silt + clay fraction does not contain sand. Given that macroaggregate-occluded silt + clay is enriched in C, it could be that silt + clay occluded within microaggregates is also enriched in C compared to free silt + clay. Some evidence supports this hypothesis (Brown et al., 2014), however these data were reported too infrequently to be included in the analysis.

4.3 Testing for carbon-saturating behavior of the silt + clay fraction

The data collected afforded an opportunity to investigate if any fraction displays C-saturating behavior between (but not within) sites as mean site-level SOC increases. If the slopes relating SOC to fraction C increased with mean site-level SOC, then we considered that fraction to show saturating behavior. (That the slopes should increase to show C saturation may be counterintuitive but is a product of having used SOC as a response variable in the original model.) Thus, for the silt + clay fraction (either occluded or free) to show evidence of C saturation, the rate of SOC increase for each unit silt + clay C concentration (g SOC kg soil^{-1} / g C kg silt + clay^{-1}) should increase with mean SOC concentration of the site. We found no evidence of this effect (Table 3), suggesting that the silt + clay fraction was not exhibiting an upper limit of C concentration in our database. We also found little evidence that rates of SOC increase with gains in total cPOM-C (kg soil^{-1}) are lower in soils with higher mean SOC concentrations, as would be expected if other compartments are C-saturated and unable to participate in SOC accumulation (Table 3). Unfortunately, no direct comparison is possible between our review data and regression coefficients generated through other approaches used to investigate C-saturation, as they rely on disrupting all aggregates to < 53 µm (or < 20 µm) and relating the soil mass in that mineral fraction to total fraction C in that fraction (Dexter et al., 2008; Feng et al., 2013; Hassink, 1997; Six et al., 2002). Stronger evidence for fraction C accumulation expected under C saturation (Six et al., 2002) may appear given alternative fractionation methods, e.g., if free POM and fine macroaggregate-occluded POM were included in the POM pool, or if POM corrections had been performed on the <53 µm fraction, which may contain very fine POM (Mueller et al., 2014) that would not be expected to show C-saturating behavior.

Our database is not equipped to explore a second tenet of C saturation theory: that increasing C inputs to soil will results in diminishing gains in SOC (Gulde et al., 2008; Novara et al., 2016; Six et al., 2002; West and Six, 2006). However, the site of Gulde et al. (2008) in this database clearly exhibits diminishing SOC response to C input, and also contains two of the highest SOC treatments (94.8 and 133.7 g kg soil^{-1}, which were removed from analyses due to data visualization challenges; see SI). When all treatments from Gulde et al. (2008) are regressed with SOC concentration as a function of free silt + clay C concentration, the slope is higher than the global slope obtained here (Table 1, 0.83 (g SOC / kg soil) / (g C / kg free silt + clay) vs 4.1 in Gulde et al. (2008)*; data not shown*), consistent with C saturation of this fraction at this very high SOC site. We form two main conclusions from the above: First, given that aggregation appears closely linked to total SOC accumulation and the stabilization and destabilization of C in the mineral fraction (Figure 6; Angst et al., 2017), it may be justified to incorporate soil aggregate dynamics alongside total clay mass and mineralogy when considering a given soil's mineral C storage capacity. Second, given the lack of clear saturating response of the silt + clay fraction in the main database (median SOC concentration = 14 g kg^{-1}; max 68) it seems unlikely that these soils are limited in SOC accumulation by the C storage capacity of the silt + clay (< 53 µm) fraction.

4.4 The SOC-dependent relationship between SOC and aggregate mass

As discussed above, soil mass was consistently redistributed into macroaggregates, away from free silt + clay and free microaggregates, as SOC increased (Figure 4, Table 1). While the direction of aggregate mass redistribution was consistent across soils, the rate of change was strongly influenced by mean site-level SOC, as evidenced by slopes different from zero (Table 3). Differential accumulation of SOC with each unit increase in macroaggregate mass meant that a standard increase of 10 g macroaggregate per 100 g soil caused a larger increase in SOC at high SOC sites (Table 4). To our knowledge this finding is unanticipated in previous literature. It may be that soils low in SOC contained lower proportions of total macroaggregate C in cPOM, representing a limited stock of C to be stabilized in microaggregates and on silt and clay surfaces. In this case, a decrease in macroaggregate turnover would result in less change in total SOC than in higher SOC soils, which generally had larger proportions of macroaggregate C as cPOM-C (SI Figure 5). In addition to providing grounds for theorizing about how mechanisms of SOC accrual operate differently between soils, these compiled data show that the benefits associated with each unit increase in SOC for soil structure (e.g., macroaggregation) is greatest in higher SOC soils.

Table 4. Effect of a 10 g increase in macroaggregate mass 100 g soil^{-1} on macroaggregate mean residence time (MRT) or / formation rate and SOC concentrations. Formation rate is related to MRT by the equation (MRT = pool [macroaggregate mass] / formation rate). Sites are grouped according to SOC concentrations. We estimated standardized higher and lower values of macroaggregate mass (plus or minus 5 g macroaggregate 100 g soil from the estimated mean macroaggregate value) within each site. We then created corresponding values of SOC change between higher and lower macroaggregate masses using site-specific slopes generated from linear hierarchical models. The % change macroaggregate MRT or formation rate within sites is estimated by the ratio of macroaggregate masses for two soils of differing SOC concentrations. Numbers in parentheses represent standard errors, generated by variability in slopes among sites.

			Given an increase in 10 g macroaggregate 100 g soil^{-1} within the same site	
SOC group	Range of mean site-level SOC, g kg soil^{-1}	Range of mean site-level macroaggregate mass, g 100 g soil^{-1}	% change in macroaggregate MRT or formation rate	Increase in SOC, g kg soil^{-1}
low	5.4 – 9.6	25.2 - 72.4	26.4 (5.3)	1.3 (0.4)
medium	10.1 – 16.1	7.3 - 76.6	53.5 (22.7)	1.9 (0.2)
high	16.4 – 55.1	22.5 - 94	21.4 (2.2)	3.6 (0.4)

4.5 Implications of soil fraction relationships with SOC for models and indicators

The study of soil fractions is connected to the search for sensitive indicators of soil change and to efforts to structure SOC models on measurable soil pools (Abramoff et al., 2017; Elliott et al., 1996; Robertson et al., 2019; Segoli et al., 2013; Stewart et al., 2008). Our review confirmed previous reports that total occluded microaggregate C shows consistent, positive relationships to total SOC (Six and Paustian, 2014). Our analysis also brought to light two caveats regarding an exclusive reliance on the occluded microaggregates as an indicator: first, total macroaggregate C showed more consistent relationships to SOC than did total occluded microaggregate C (R^2M 0.85 vs 0.62; Table 1). As occluded microaggregate mass and C are constituents of macroaggregates, any changes in occluded microaggregate C or mass are necessarily captured by the macroaggregate fraction. We were not able to derive measures of variance around SOC content or aggregate mass, so the question of whether macroaggregate or occluded microaggregate are associated with different measurement variability remains open. Second, occluded microaggregate C and mass as a percentage macroaggregate C or mass was not a reliable indicator of SOC change, because C and mass occluded within macroaggregates was not consistently distributed to either occluded microaggregates or cPOM with increases in SOC. Our review provides support for further development of fraction/aggregate-based SOC models, and for attention to be given to enacting nested processes of macroaggregate C occlusion and C stabilization. Given the data available for this review, our findings are most relevant to the top ~30 cm of soil, with uncertain application to deep SOC, and may have limited application to permafrost-affected or peat soils. For topsoils at moderate latitudes, equipping SOC models with the ability to simulate macroaggregate dynamics may provide a path forward for efforts to simulate SOC dynamics.

5. Conclusions

We present the first quantitative review of macroaggregate-occluded fractions and their relation to SOC concentration. Consistent with previous understandings, a redistribution of soil mass toward macroaggregates is associated with an increase in SOC concentration. Mechanisms linking increased macroaggregate mass (and reduced macroaggregate turnover) appear attributable in part to the role of macroaggregates in increasing C stabilization and/or protection in occluded microaggregates and silt + clay. Dynamics of silt + clay accumulation with SOC between sites did not indicate that the silt + clay fraction was displaying a strict upper limit of C storage capacity, despite the inclusion of observations with SOC concentrations > 60 g SOC kg soil^{-1}. Our review revealed consistent proportions of SOC accumulation occurred in C macroaggregates and occluded microaggregates. These relationships have potential to be leveraged as benchmarks for fraction-based SOC models and invite an improved understanding of mechanisms governing soil macroaggregate formation and disruption.

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Figure captions

Figure 1. Soil fractionation scheme common to studies included in the review. Light gray fractions are non-occluded, while dark gray fractions are occluded (within macroaggregates). Size classes following Elliott (1986) and Six et al. (2000a).

Figure 2. All observations for the relationship between total SOC (g kg soil^{-1}) and total fraction C (g fraction C kg soil^{-1}) for six soil fractions. Lines represent individual site-level regressions generated by linear hierarchical models. All data points are represented by a filled gray circle (symbols appear darker are due to overlap in data points), and global slopes are reported in Table 1.

Figure 3. All observations for the relationship between aggregate C concentrations (g aggregate C kg aggregate^{-1}) and total SOC (g kg soil^{-1}) for six aggregate fractions. Lines represent individual site-level regressions generated by linear hierarchical models. All data points are represented by a filled gray circle (symbols appear darker are due to overlap in data points).

Figure 4. All observations for the relationship between aggregate mass (g aggregate 100 g soil^{-1}) and total SOC (g kg soil^{-1}) for six aggregate fractions. Lines represent individual site-level regressions generated by linear hierarchical models. All data points are represented by a filled gray circle (symbols appear darker are due to overlap in data points).

Figure 5. All observations for the relationships: **A)** total microaggregate mass (g free microaggregate + occluded microaggregate 100 g soil^{-1}) and macroaggregate mass, y = 0.06x + 55.0, R^2M = 0.01, CI = -0.11, 0.24; **B)** SOC g kg soil^{-1} and total microaggregate mass, y = 0.08x + 15.5, R^2M = 0.01, CI = -0.04, 0.20 and **C)** SOC and the percent total microaggregate occluded in macroaggregates, y = 0.21x + 10.7, R^2M = 0.14, CI = 0.14, 0.28. Lines represent individual site-level regressions generated by linear hierarchical models. All data points are represented by a filled gray circle (symbols appear darker are due to overlap in data points).

Figure 6. Relative concentrations of occluded and free **(A)** silt and clay and **(B)** microaggregates. Solid black lines represent 1:1 lines. Dashed gray lines represent linear regression through all points. Regression coefficients: **(A)** y = 1.29x + 0.47, R^2 = 0.68; **(B)** y = 1.2x + 1.6; R^2= 0.93. The total C supplied by the enrichment of these fractions was moderate on a whole soil basis, representing 2.1% of total SOC from occluded silt + clay and 6.7% of total SOC from occluded microaggregates on average.

Figure 7. Mass of macroaggregate per 100 g soil as related to **(A)** mean annual precipitation (MAP) and **(B)** percent clay. Only observations from the top layer of soil reported. Linear regressions represent: **(A)** regression through all points (solid line, $y = 0.02x + 34.9$, $p < 0.0001$, $R^2 = 0.16$); regression through all points excluding MAP > 3,000 (dot-dash line, $y = 0.02x + 32.9$, $p < 0.0001$, $R^2 = 0.13$); boundary-line analysis, excluding MAP > 3,000 (dotted line, $y = 0.05x - 11.8$, $p < 0.0001$, $R^2 = 0.89$). **(B)** Regression through all points ($y = 0.27x + 45.6$, $p = 0.01$, $R^2 = 0.03$).

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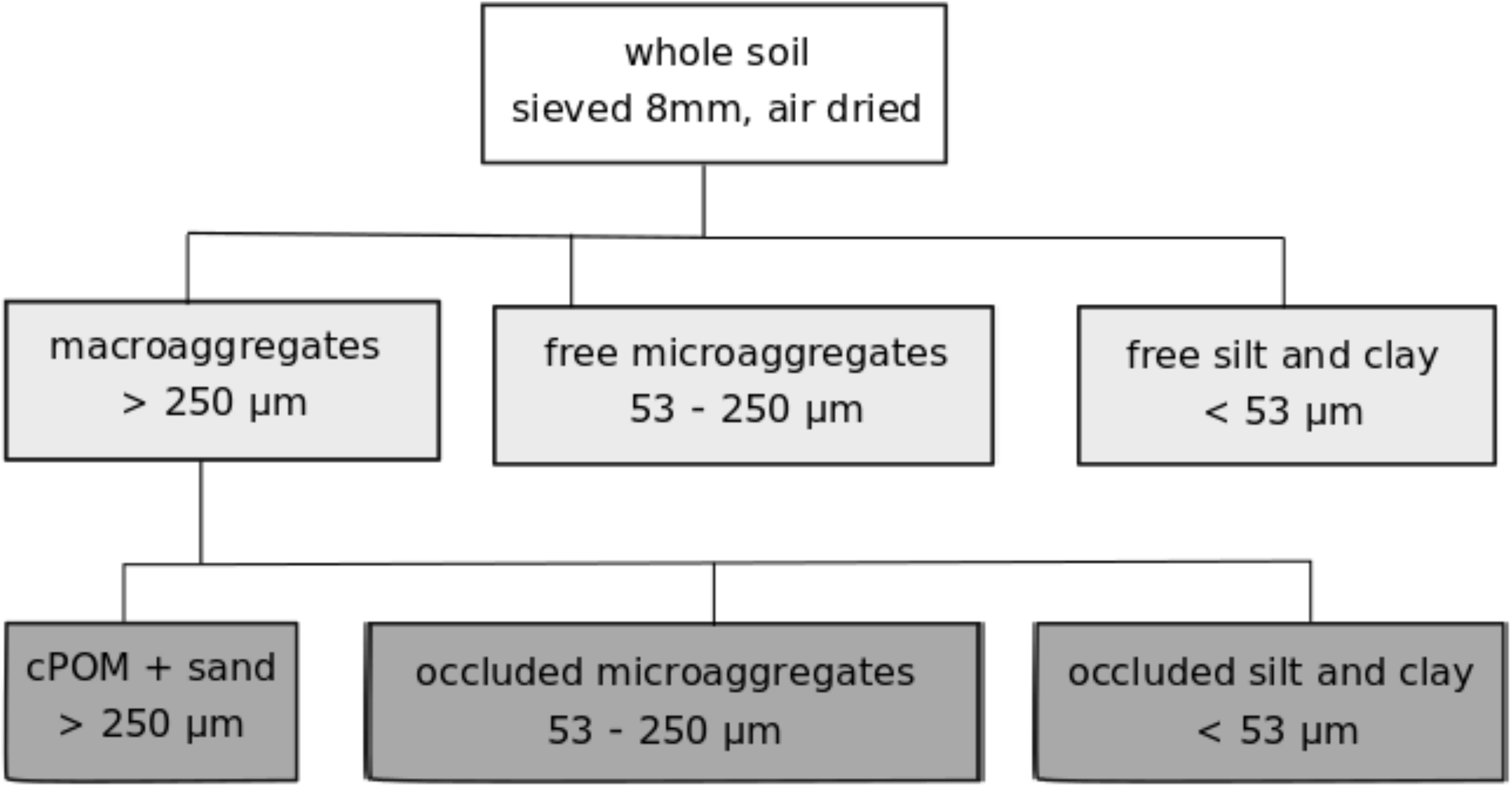
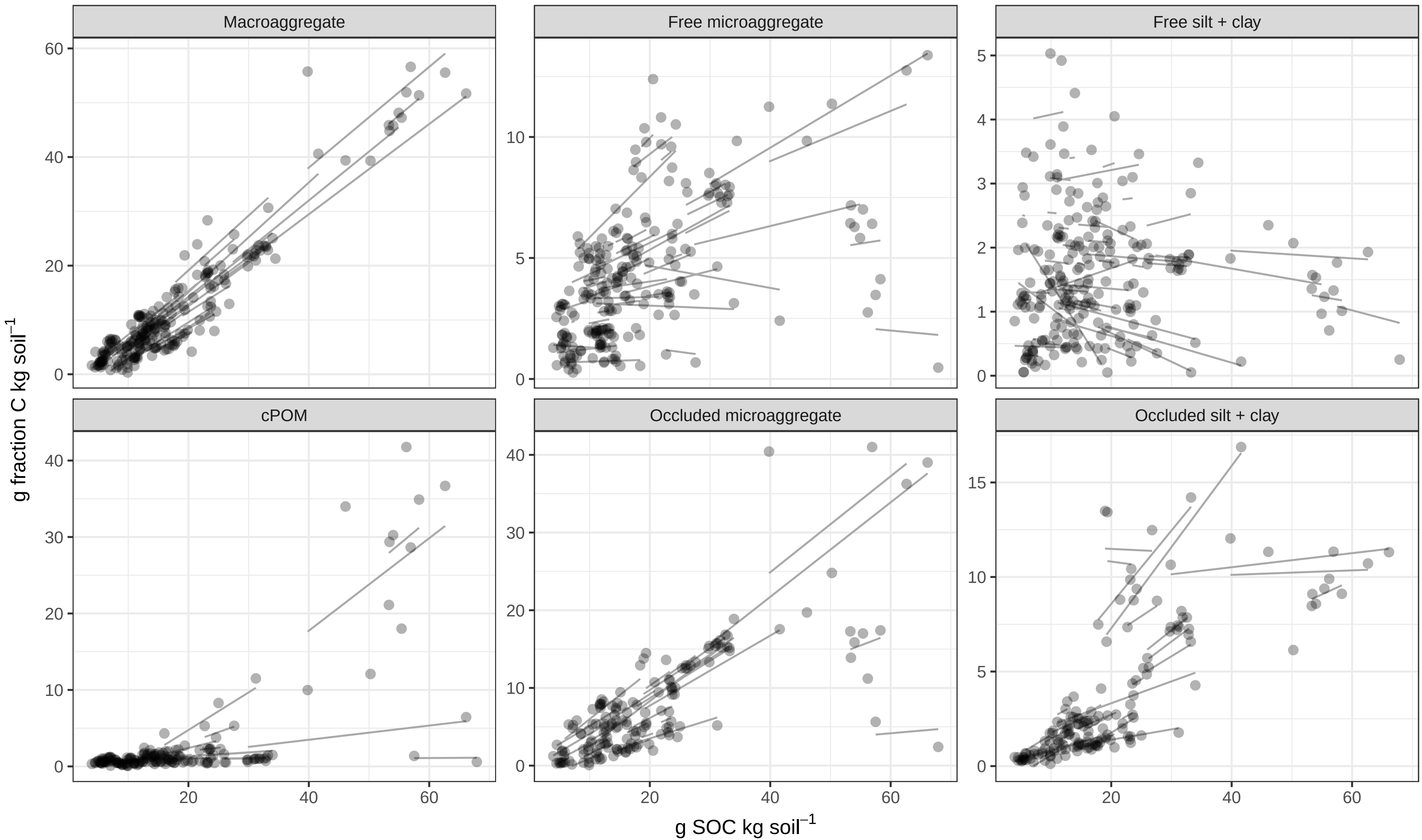

whole soil
sieved 8mm, air dried
macroaggregates
> 250 µm
free microaggregates
53 - 250 µm
free silt and clay
< 53 µm
cPOM + sand
> 250 µm
occluded microaggregates
53 - 250 µm
occluded silt and clay
< 53 µm

Figure 2



Macroaggregate
Free microaggregate
Free silt + clay
cPOM
Occluded microaggregate
Occluded silt + clay
g fraction C kg soil^{-1}
g SOC kg soil^{-1}
0
20
40
60
0
5
10
1
2
3
4
5
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Figure 3

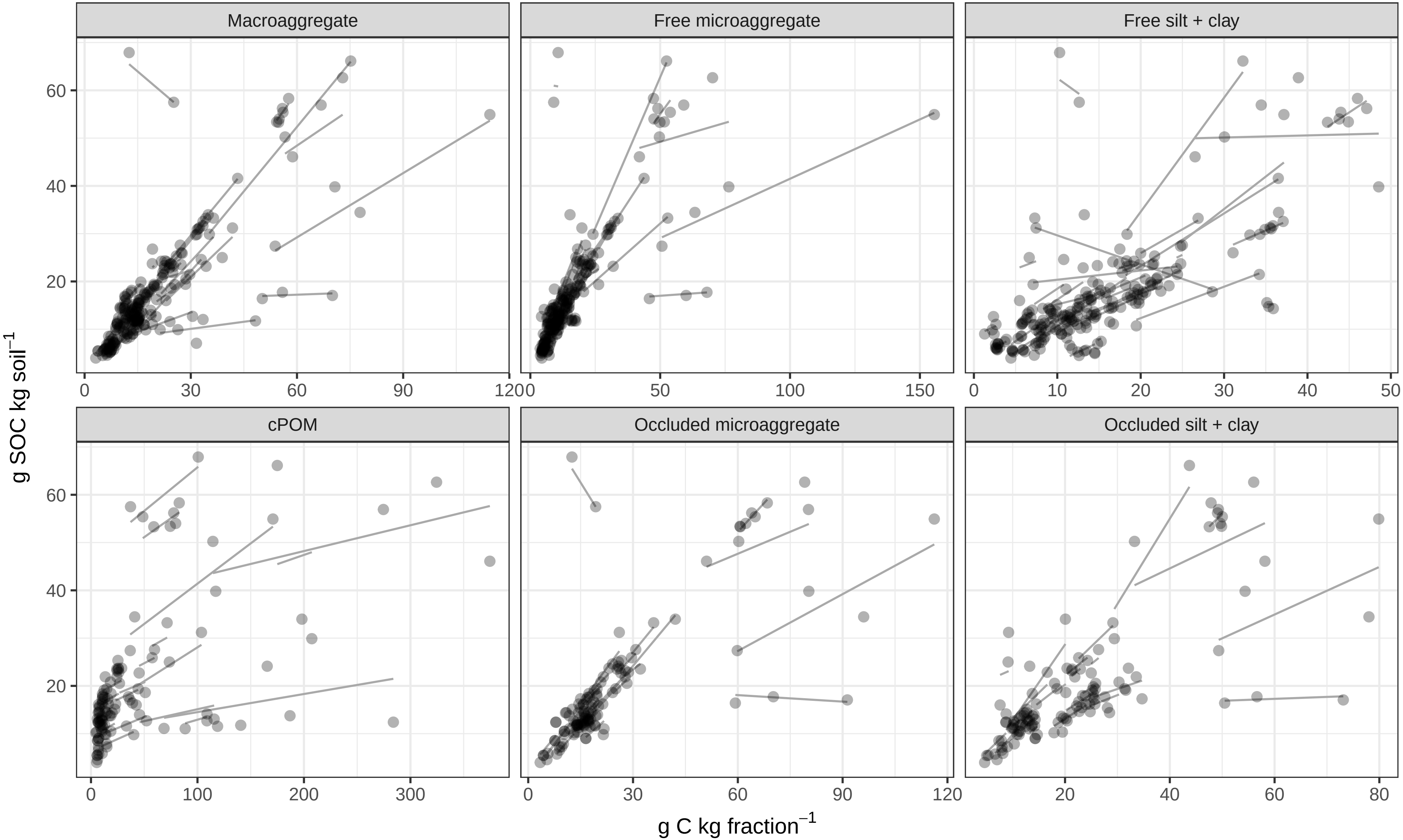
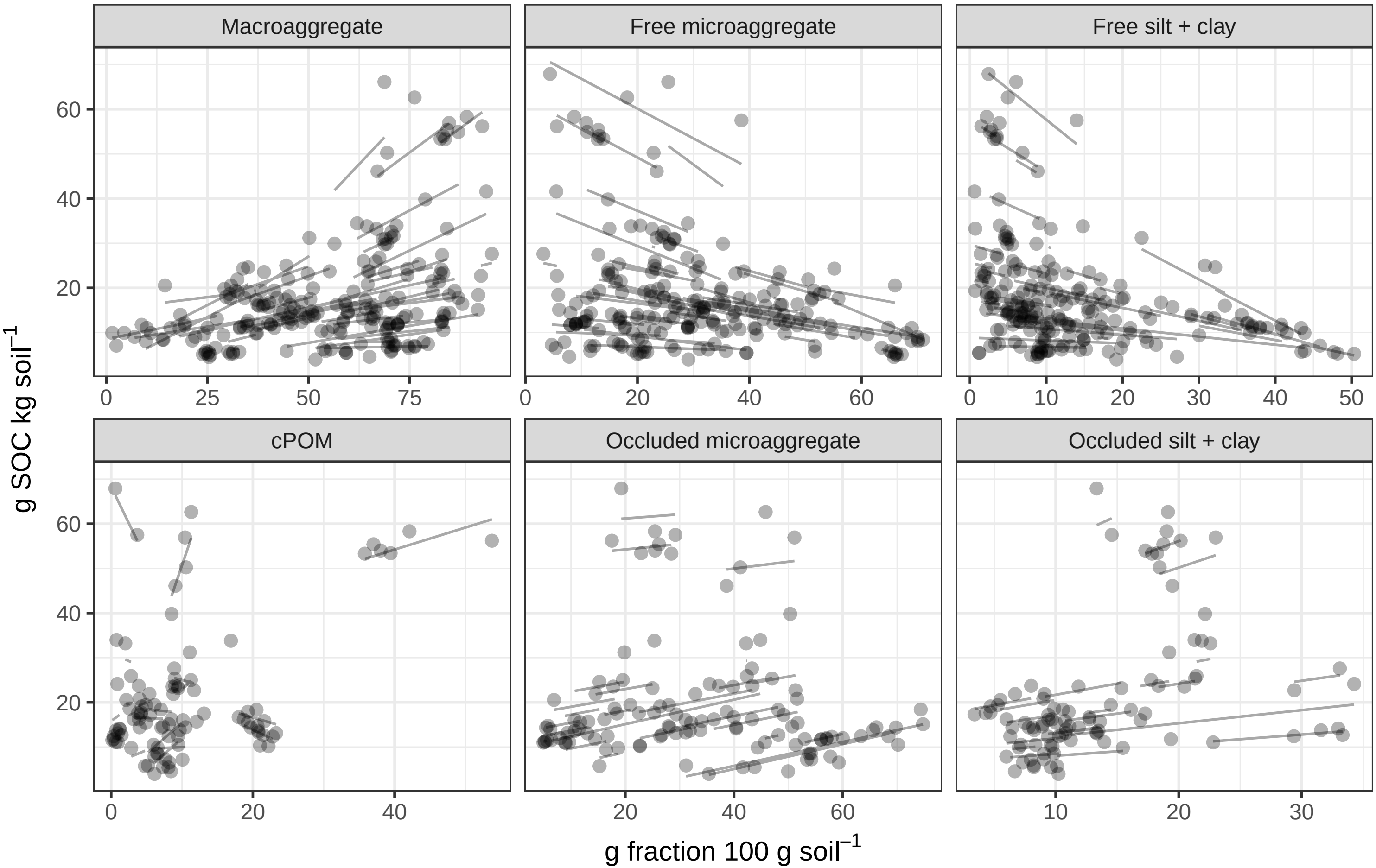

Macroaggregate
Free microaggregate
Free silt + clay
cPOM
Occluded microaggregate
Occluded silt + clay
g SOC kg soil^{-1}
g C kg fraction^{-1}
0
20
40
60
30
90
120
50
100
150
10
300
200
80

Figure 4


Macroaggregate
Free microaggregate
Free silt + clay
cPOM
Occluded microaggregate
Occluded silt + clay
g SOC kg soil^{-1}
g fraction 100 g soil^{-1}
0
25
50
75
0
20
40
60
0
10
20
30
40
50
0
20
40
20
40
60
10
20
30
20
40
60

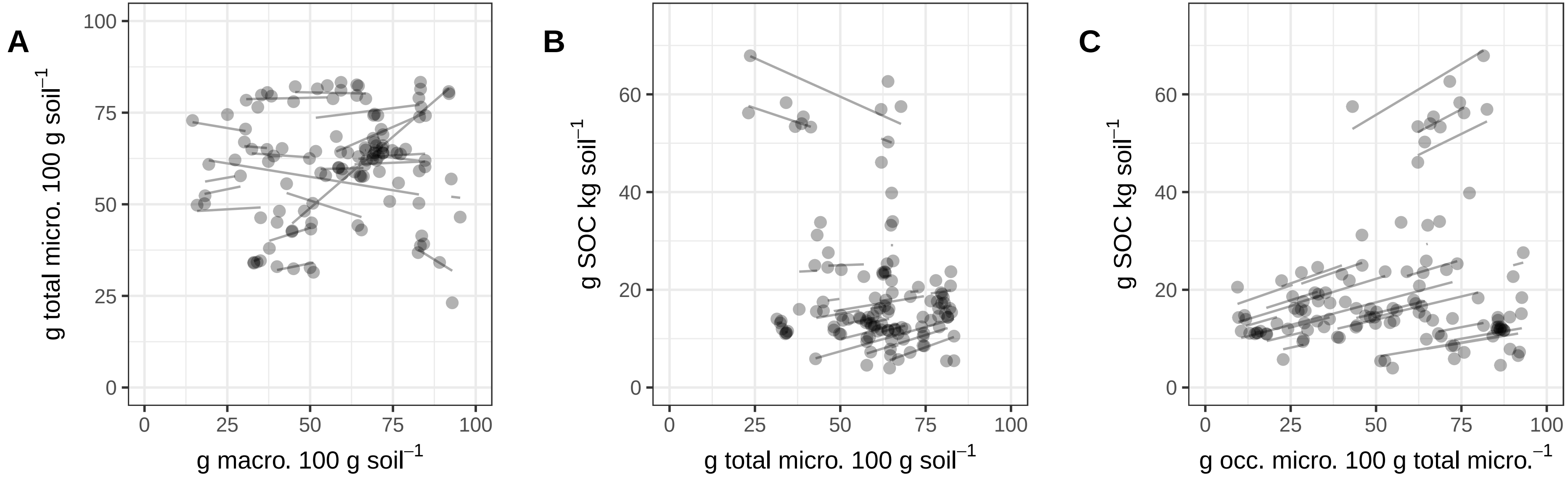
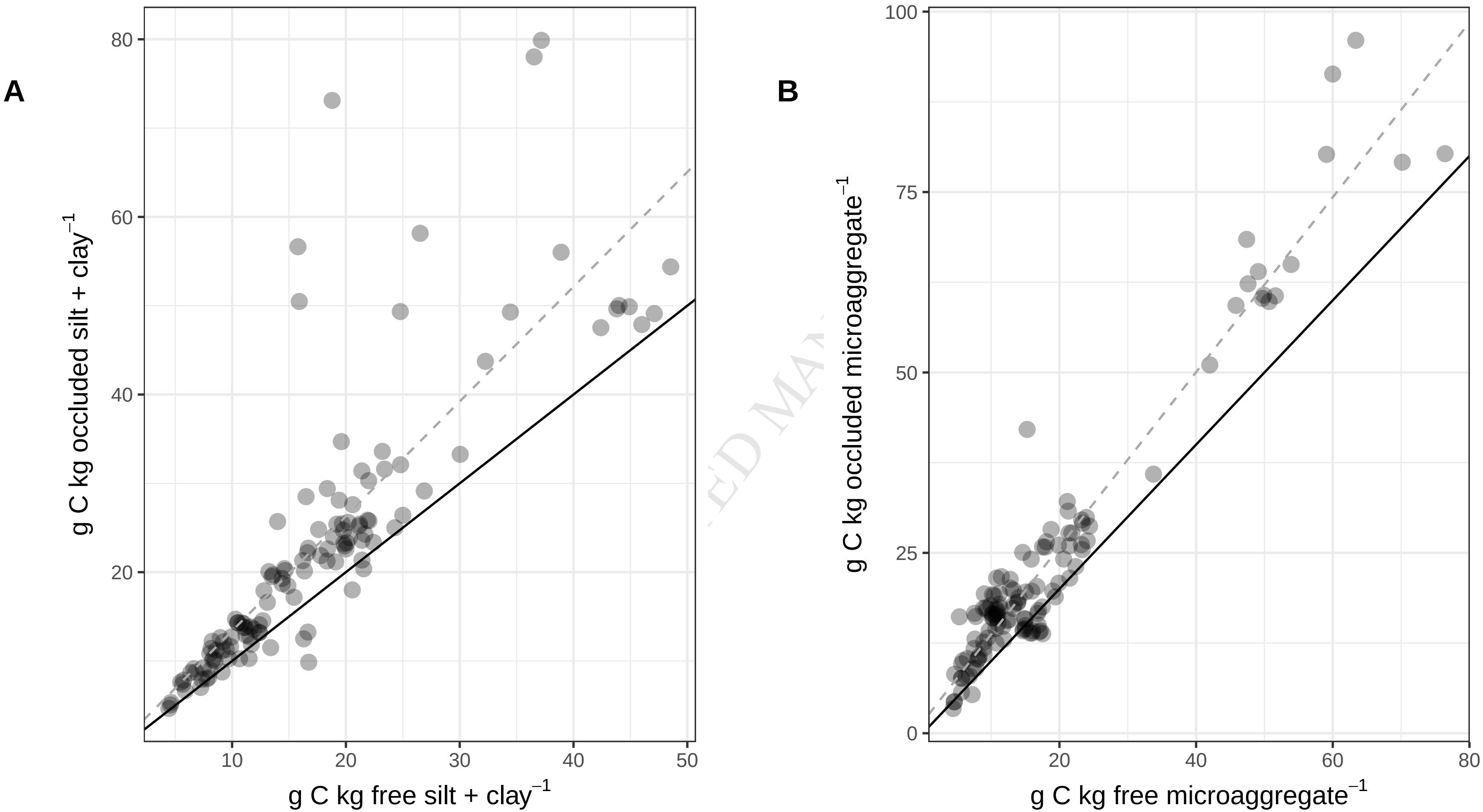
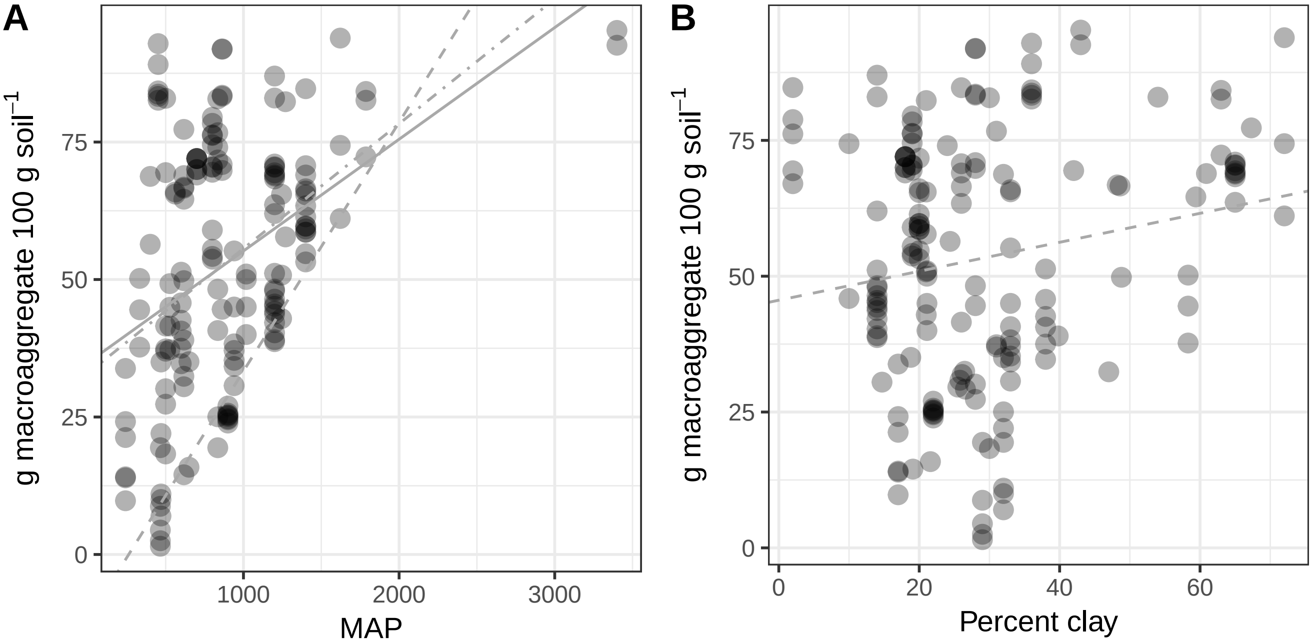

A
g total micro. 100 g soil^{-1}
g macro. 100 g soil^{-1}
0
25
50
75
100
B
g SOC kg soil^{-1}
g total micro. 100 g soil^{-1}
0
20
40
60
C
g SOC kg soil^{-1}
g occ. micro. 100 g total micro.$^{-1}$

Figure 6


A
80
60
40
20
g C kg occluded silt + clay^{-1}
10
20
30
40
50
g C kg free silt + clay^{-1}
B
100
75
50
25
0
g C kg occluded microaggregate^{-1}
20
40
60
80
g C kg free microaggregate^{-1}



A
B
g macroaggregate 100 g soil^{-1}
75
50
25
0
1000
2000
3000
MAP
0
20
40
60
Percent clay

- We quantitatively reviewed 41 studies reporting macroaggregate-occluded fractions
- SOC increase was associated with gains in macroaggregate mass
- Macroaggregate-occlusion increased C concentration of microaggregates, silt + clay
- Macroaggregates may enable stabilization of POM to microaggregates, silt + clay
- 83% of SOC accumulated in macroaggregates; 43% occurred in occluded microaggregates