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Drivers of the amount of organic carbon protected inside soil aggregates estimated by crushing: A meta-analysis

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ABSTRACT

Given the importance of soil organic carbon (SOC) stocks and their dynamics in the regulation of climate change, understanding the mechanisms of SOC protection from decomposition is crucial. It is recognized that soil aggregates can provide effective protection of organic carbon from microbial decomposition. Currently, there is no systematic method for estimating the amount of protected carbon within aggregates. However, differences between CO₂ emissions from incubation of intact versus crushed aggregates have been widely used as a proxy for SOC physical protection within aggregates. There is no global analysis on this type of experiment yet, nor on the drivers of the amount of SOC physically protected in soils. Using a meta-analysis including 165 pairs of observations from 22 studies encompassing a variety of ecosystems, climate and soil types, we investigated the crushing effects on cumulative carbon mineralization from laboratory incubation experiments. The aggregates were initially separated by either wet sieving or dry sieving before dry crushing. Our results indicated that aggregate crushing led on average to +31 % stimulation of carbon mineralization compared with intact aggregates, which represented 0.65 to 1.01 % of total SOC. This result suggests the mineralization of a previously protected pool of labile organic carbon. The linear regression analysis showed that the crushing effect on carbon mineralization depended on soil characteristics (carbon content, clay content and pH) as well as on aggregate size. Crushing aggregates stimulated carbon mineralization relative to control, up to +63 % in large aggregates (>10 mm), +38 % in large macro-aggregates (2–8 mm), +14 % in small macro-aggregates (0.25–2 mm) and +54 % in micro-aggregates (<0.25 mm). Within each aggregate size-class, the crushing effect depended on the crushing intensity. The destruction of aggregates to <0.05 mm size had a greater effect on carbon mineralization (+130–133 %) than the destruction of aggregates to >2 mm (+3 to 40 %), <2 mm (+58 to 62 %) and <0.25 mm (+32 to 62 %) sizes regardless of the initial aggregate size. These results suggest that macroaggregates (>0.25 mm) are less protective than microaggregates (<0.25 mm). Our dataset also show that soil physico-chemical characteristics and experimental conditions influenced more the amount of protected SOC than land use and management. Contrary to our expectations the crushing effect was not affected by tillage practices nor land use. Standardizing the experimental conditions of aggregate crushing and subsequent incubation is needed to assess and compare the amount of physically protected SOC in diverse soils, and then to better understand the processes and drivers of SOC protection inside aggregates.

1. Introduction

Given the importance of soil organic carbon (SOC) stocks and their dynamics in the regulation of climate change, understanding the mechanisms of SOC protection from decomposition is crucial. Soil

organic carbon decomposition is affected by its biochemical composition and it can be stabilized against decomposition by its association with minerals and by its physical protection in the soil structure (Sollins et al., 1996; Baldock and Skjemstad, 2000; Six et al., 2002; von Lützwow et al., 2006).

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The observed increase in SOC mineralization following the crushing of soil aggregates was early interpreted (Rovira and Greacen, 1957) as an expression of SOC physical protection, i.e. that the spatial arrangement of soil particles can hinder biodegradation. Indeed, biodegradation requires direct contact between microbial decomposers or their exoenzymes and the organic substrate, and the low density of microorganisms on the one hand and the complex three-dimensional arrangement of soil particles and voids on the other hand cause a spatial disconnection at the microscale (Chenu and Stotzky, 2002; Dungait et al., 2012). In addition, the availability of oxygen to microbial decomposers may be limiting in some locations within the soil structure as it was demonstrated by directly measuring oxygen concentration in soil aggregates using needle type optodes (e.g. Sexstone et al., 1985; Parry et al., 1999). Spatial disconnection and oxygen limitation, are both alleviated when crushing soil aggregates (Balesdent et al., 2000).

Research on the physical protection of soil organic carbon has historically focused on soil aggregates, i.e., small volumes of soil within which the cohesion between particles is larger than that with the adjacent aggregates, so that they can be easily separated and organic carbon quantified therein. Water-stable aggregates were assumed to represent sites of physical protection of SOC because the life-expectancy of water-stable aggregates is expected to be longer than that of water-unstable ones (Plante and McGill, 2002). Hence, any spatial arrangement of soil particles physically protecting organic carbon from biodegradation should persist longer in water-stable aggregates than in unstable ones (Balesdent et al., 2000, Plante and McGill, 2002). The distribution of organic carbon in soil aggregates has been extensively studied (Tisdall and Oades, 1982; Elliott, 1986; Gupta and Germida, 1988; Angers, 1992; Beare et al., 1994b). Combining aggregates fractionation with ^{13}C natural abundance showed that the turnover of soil organic carbon differed between aggregate sizes (Puget et al., 1995; Angers and Giroux, 1996; Puget et al., 2000; Six et al., 2000; Six et al., 2004) and that organic carbon located within or outside soil aggregates exhibited contrasted dynamics (Golchin et al., 1994; Besnard et al., 1996; Six et al., 2000). Particulate organic matter (POM) inside aggregates turned over more slowly (40–49 years) compared to POM outside aggregates (19–27 years) (Golchin et al., 1995). The primary site of SOC protection appears to be particularly located in microaggregates (<0.25 mm) with a SOC turnover of 209 ± 95 years compared to a SOC turnover in macroaggregates (>0.25 mm) of 42 ± 18 years (Six and Jastrow, 2002).

While physical protection of SOC was demonstrated *in situ* by comparing the turnover rate of SOC with different locations in the soil structure, there is no widely agreed method to assess the amount of organic carbon physically protected in soils. Physically protected SOC pools are often estimated from the amount of particulate organic carbon or SOC located in aggregate fractions (von Lütow et al., 2006). Incubation experiments of intact versus crushed aggregates have also been widely used as a proxy for physical protection within aggregates, with contrasted results (Crasswell and Waring, 1972; Elliott, 1986, Gupta and Germida, 1988; Gregorich et al., 1989; Beare et al., 1994a, Balesdent et al., 2000; Chevallier et al., 2004; Chevallier et al., 2011). Analyzing the results of such experiments should bring information on the drivers of the amount of physically protected SOC.

According to the model of Tisdall and Oades (1982), microaggregates provide the greatest protection, whereas macroaggregates which are built up of microaggregates are less effective. Therefore, we can assume that the initial size of the aggregates and the crushing intensity must influence the amount of deprotected carbon. Indeed, several studies showed that the crushing effect increased as the crushing was more intense, i.e., resulted in finer aggregate sizes (Balesdent et al., 2000). Soil texture and soil organic matter content are considered as a major drivers of the extent of physical protection of organic matter in soils (Balesdent et al., 2000). Indeed, soil organic matter and clay-sized particles are the main binding agents in the formation and stabilization of aggregates, along with Fe and Al oxides and hydroxides in particular in oxisols and with carbonates in carbonated soils (Tisdall

and Oades, 1982; Amézqueta, 1999; Chenu et al., 2000; Duchicela et al., 2012; Portella et al., 2012). We expect hence that crushing the aggregates of a fine-textured soil would lead to a greater response than a coarse-textured soil, while opposite effects were also found (Raza-fimbelo et al., 2013), as aggregation was likely provided by Al-containing crystalline sesquioxide in the studied soils.

Land use and agricultural practices are known to largely influence SOC stocks and dynamics, as a result of either or both increased organic carbon inputs to soil and organic carbon outputs by mineralization (Chenu et al., 2019). More physical protection in grassland soils compared to cropland soils and in no-till soils compared to conventionally tilled soils is generally considered to explain larger SOC stocks in the former (Six et al., 2000). Six et al. (1999) as well as Puget et al. (1995) observed higher SOC contents in aggregates in no-till soils than in conventionally tilled soils. No tillage improves macroaggregate stability and microaggregate formation, which better protects carbon from microbial decomposition (Jastrow et al., 1996; Six et al., 2000). Therefore, we expect to observe a larger crushing effect on soil from undisturbed systems (forest, grassland, no-till) compared to regularly tilled ones.

The incubation experiments of intact versus crushed aggregates is the method often used to estimate the amount of organic carbon physically protected in soils. This approach has been applied in a substantial number of publications making relevant to analyze this corpus of literature jointly in a *meta-analysis*. The objectives of this *meta-analysis* were on the one hand to assess whether and to which extent crushing soil aggregates caused a flush of mineralization and on the other hand to establish possible relationships between the crushing effect and factors expected to increase physical protection. We hypothesized that the crushing effect would be more important: (i) when finer sized aggregates are disrupted, (ii) as clay and SOC content increases, and (iii) in undisturbed systems (forest, grassland, no tillage) compared to frequently disturbed ones (conventional tillage). We used a *meta-analytical* approach with a random effects model accounting for the non-independence of multiple observations extracted from the same study (Curtis and Queenborough, 2012) to test our hypotheses and evaluate how soil characteristics modulate the effect of crushing on SOC mineralization.

2. Materials and methods

2.1. Data acquisition and selection

We searched for relevant articles in Web of Science, Google Scholar and Scopus using the following search terms: “crush”, “grinding”, “ground”, “aggregate”, “intact”, “carbon mineralization”, “organic matter”, “physical protection”, and a combination of these terms. We also searched for articles that were cited in the publications we found. We focused the analysis on aggregates of different sizes. The aggregate size has been classified according to the ranking commonly found in the literature (Cambardella and Elliott, 1992; Six et al., 2000; Márquez et al., 2004) into large aggregate (>10 mm), large macro aggregate (2–8 mm), small macroaggregate (0.25–2 mm) and microaggregate (<0.25 mm). All articles used from these studies met the following criteria: (i) experiments had a control treatment with “intact aggregates” of different sizes; (ii) aggregates were dry crushed with mortar or mechanical mill, and then passed to different sieve sizes (Fig. 1). The initial separation of the aggregates was performed either by wet sieving or by dry sieving (Table 1). According to these criteria, a total of 22 studies from 22 sites were included (Fig. 2), providing 165 coupled observations (intact aggregates vs crushed aggregates) of cumulative carbon mineralization. Data from the figures were extracted using the WebPlotDigitizer (Huwaldt, 2013; Burda et al., 2017) digitizing software to convert the data points into numerical values.

For each study site, we collected information on ecosystems type (forest soil, grassland, cropland or fallow soil), soil management (no-

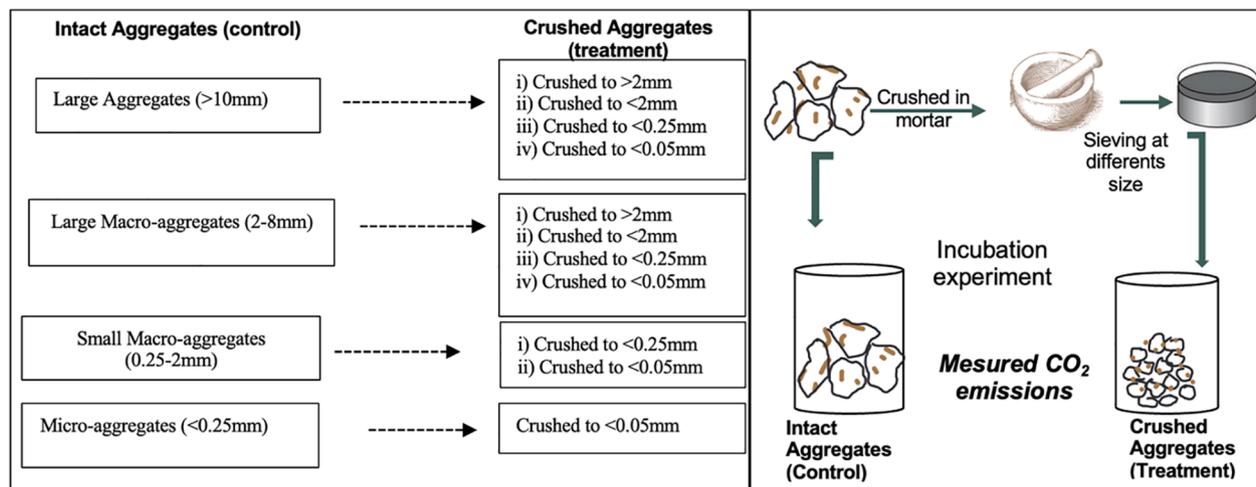


Fig. 1. Illustration of the selection criteria (control and treatment) of retrieved studies, showing soil aggregates types and experiment protocols.

tillage vs tillage), crop management, climate (temperate, tropical, Mediterranean and boreal) and soil texture expressed in percentage of clay, silt and sand. Soil texture classes were then grouped into three categories: coarse texture (sand and loamy sand soils), medium texture (loam, silty loam and clay loam) and fine texture (sandy clay loam, silty clay loam, clay loam, clay), according to the soil survey manual of the USDA (Canarini et al., 2017). The SOC contents of intact and crushed aggregates were considered to calculate the specific cumulative carbon mineralization. We also collected soil pH data within each study.

Information about incubation conditions were collected: incubation length expressed in days, incubation temperatures in degrees Celsius (°C) and soil moisture data. Soil moisture data were mostly expressed in gravimetric water content, but also as volumetric water content, percentage of water holding capacity (%WHC), percentage of water filled pore space (%WFPS) or matric potential (MPa).

2.2. Data analysis

To evaluate the effects of aggregate crushing between different studies, the response was normalized, expressing the effect relative to the control (mineralization of intact (not crushed) aggregates). When only the daily mineralization rate was available, we calculated the cumulative mineralization using the following formula:

$$C_{j+1} = \frac{R_j + R_{j+1}}{2} \times (t_{j+1} - t_j) + C_j \quad (1)$$

(Lin et al., 2015; Zhang et al., 2020) where C_j is the cumulative amount of mineralized carbon from the start of the incubation to the day of measurement “j” expressed in mg C kg⁻¹ soil; R_j is the rate of mineralization in mg C kg⁻¹ soil d⁻¹; “t” is the incubation time in days (d). If j = 0, $R_j = 0$, $C_j = 0$.

To assess physical protection, we used the relative increase in mineralization, rather than the difference of mineralized carbon between the crushed and intact treatment. This choice allowed us to homogenize all the data and to express the results in response ratio (RR). The RR is defined as: $RR = (X_T/X_C)$ (Gurevitch et al., 2001; Canarini et al., 2017; Zhang et al., 2020) where X_T and X_C are the mean cumulative carbon mineralization values of the crushed treatment (T) and the control (intact) group (C). For each experiment, the crushing effect (LnRR) was calculated as the natural logarithm (Ln) of the response ratio (RR). There are two reasons for using the natural logarithm. The first is that the logarithm linearizes the data by giving equal weight to the numerator and denominator of the RR which will therefore have the same influence on the deviation of the results. The second reason is that LnRR values are more normally distributed than RR values for small

samples (Hedges et al., 1999). If $RR < 1$, a negative crushing effect on carbon mineralization was observed and if $RR > 1$ the crushing led to an increased carbon mineralization. Therefore, a negative LnRR indicates that aggregates crushing reduces the carbon mineralization relative to the intact aggregates (control), whereas a positive LnRR indicates that crushing increases the carbon mineralization relative to the intact aggregates. Assuming that crushed treatment and control were independent, the variance of LnRR was calculated as:

$$Var(\ln RR) = Var(\ln C - \ln T) = \frac{SD_C^2}{n_C \bar{X}_C^2} + \frac{SD_T^2}{n_T \bar{X}_T^2} \quad (2)$$

(Hedges et al., 1999) where SD is the standard deviation and “n” is the sample size of the mean response values. Mean effect (LnRR) was calculated using random-effects model because there is true variation in effect sizes among studies (Lajeunesse, 2013; Zhang et al., 2020). The within variance (Var) and between-study variance (τ^2) were used in the random-effect model to calculate the weighting factor [$1/(Var + \tau^2)$]. Between-study variance (τ^2) was calculated using restricted maximum likelihood (REML) estimator as it is better for continuous data (Veroniki et al., 2016; Zhang et al., 2020). We used these estimates to calculate I^2 , indicating the proportion of total variance due to true heterogeneity among effect sizes (Higgins and Thompson, 2002) and identifying low (25%), medium (50%) and high (>75%) heterogeneity (Huedo-Medina et al., 2006). In simple terms, a low I^2 indicates that variability among effect sizes is mainly due to sampling error within studies, while a high I^2 indicates that variability is caused by true heterogeneity among studies (Canarini et al., 2017). Multiple imputation was used to derive standard deviations for observations not reporting this (70 in total among all variables). First, coefficients of variation were calculated for each observation with reported standard deviations, dividing the standard deviation by the reported mean for LnRR. Missing coefficients of variation were then imputed by random sampling with replacement, either from the total data set. Finally, each imputed value was converted to standard deviation by multiplying with the reported mean of the imputed observation, allowing all observations to be included in the following meta-analysis. This procedure was repeated 1000 times, and final parameter estimates were obtained as the average across runs. The 95% confidence interval (CI) was calculated by variance weighted bootstrapping (999 iterations) (Huo et al., 2017). Heterogeneity was explained with different moderators (including categorical variables) by Q statistics, and the subgroup analysis was used for categorical variables.

In order to show the crushing effects more clearly, LnRR was transform back to the percentage change (% Change) relative to control by the formula $(e^{\ln(RR)} - 1) * 100\%$ (Canarini et al., 2017; Zhang et al.,

Table 1
Comparison of the effect of aggregate crushing on C mineralization in different studies corresponding to different site and soil characteristics and experimental conditions. SOC: Soil Organic Carbon; WHC: Water Holding Capacity; WFPS: Water Free Pore Space.

Publications	Climatic information	Land use	Practices	Soil depth (cm)	pH	Clay content (%)	Soil texture	SOC (%)	Method of initial aggregate size separation	Initial aggregate size (mm)	Crushing operation	Crushed to (mm)	With inert sand or no sand	Moisture	Temperature (°C)	Time (days)	Carbon mineralization Crushed/Intact (RR)	Crushing effect (LnRR)	% Change in carbon Mineralization
Aoyama et al.,1999	Temperate	Cropland	Tillage	0–10	6.2	24	Loam	2.60	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	–50 KPa	25	21	1.27	0.24	27
Beare et al., 1994a	Mediterranean	Cropland	Tillage	0-5	6.78	21	Sandy Clay Loam	1.23	Wet sieving	>2	Mortar crushed + sieving	<2	Sand	55 % WFPS	24	20	1.11	0.11	11
	Mediterranean	Cropland	No Tillage	0-5	7.05	21	Sandy Clay Loam	1.76	Wet sieving	>2	Mortar crushed + sieving	<2	Sand	55 % WFPS	24	20	1.24	0.22	24
	Mediterranean	Cropland	Tillage	0-5	6.78	21	Sandy Clay Loam	1.23	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	55 % WFPS	24	20	1.18	0.17	18
	Mediterranean	Cropland	No Tillage	0–5	7.05	21	Sandy Clay Loam	1.76	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	55 % WFPS	24	20	1.29	0.25	29
Bischoff et al., 2017	Boreal	Cropland	Fodder crops	0–10	7.6	36.3	Silt Loam	4.29	Dry sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	60 % WHC	20	28	1,04	0.03	4
	Boreal	Cropland	Tillage	0–10	7.1	23.6	Loam	1.57	Dry sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	60 % WHC	20	28	0.95	–0.05	–5
	Boreal	Grassland	Extensive pasture	0–10	7.6	36.3	Silt Loam	3.81	Dry sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	60 % WHC	20	28	1.03	0.03	3
Bossuyt et al., 2002	Temperate	Cropland	Tillage	0–2.25	7.1	21	Sandy Clay Loam	1.64	Wet sieving	0.053–0.25	Mortar crushed + sieving	<0.053	No Sand	55 % WFPS	30	21	1.40	0.34	40
	Temperate	Cropland	No Tillage	2.5–5	6.8	21	Sandy Clay Loam	1.39	Wet sieving	0.053–0.25	Mortar crushed + sieving	<0.053	No Sand	55 % WFPS	30	21	1.58	0.46	58
	Temperate	Cropland	Tillage	5–15	6.8	21	Sandy Clay Loam	0.75	Wet sieving	0.053–0.25	Mortar crushed + sieving	<0.053	No Sand	55 % WFPS	30	21	2.40	0.80	124
	Temperate	Cropland	Tillage	0–2.25	7.1	21	Sandy Clay Loam	0.93	Wet sieving	>0.25	Mortar crushed + sieving	<0.25	No Sand	55 % WFPS	30	21	1.17	0.16	17
	Temperate	Cropland	No Tillage	0–2.25	7.1	21	Sandy Clay Loam	1.58	Wet sieving	>0.25	Mortar crushed + sieving	<0.25	No Sand	55 % WFPS	30	21	1.19	0.18	19
Chevallier et al., 2004	Tropical	Grassland	Restored pasture	0–30	6.5	56	Clay	2.36	Dry sieving	10–20	Mortar crushed + sieving	<5	No Sand	75 % WHC	28	21	1.44	0.35	42
Chevallier et al., 2011	Tropical	Cropland	Tillage	0–30	7	54	Clay	2.05	Dry sieving	<2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.40	0.34	40
Curtin et al., 2014	Temperate	Cropland	Tillage	5–10	6	40	Clay Loam	3.14	Dry sieving	>4	Sieving	<4	No Sand	–10 KPa	25	15	1.00	0.00	0
	Temperate	Cropland	Tillage	5–10	6	40	Clay Loam	2.30	Dry sieving	2–4		<0.25	Sand	–10 KPa	25	30	1.59	0.46	59

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Table 1 (continued)

Publications	Climatic information	Land use	Practices	Soil depth (cm)	pH	Clay content (%)	Soil texture	SOC (%)	Method of initial aggregate size separation	Initial aggregate size (mm)	Crushing operation	Crushed to (mm)	With inert sand or no sand	Moisture	Temperature (°C)	Time (days)	Carbon mineralization Crushed/Intact (RR)	Crushing effect (LnRR)	% Change in carbon Mineralization
D'Angelo et al., 2009 Elliott, 1986	Temperate	Cropland	Minimum Tillage	0–15	6	16	Silt Loam	2.57	Dry sieving	>4	Mortar crushed + sieving Sieving	<4	No Sand	–10 KPa	25	14	1.05	0.04	4
	Temperate	Grassland	Pasture	0–15	6	16	Silt Loam	3.09	Dry sieving	>4	Sieving	<4	No Sand	–10 KPa	25	14	1.04	0.04	4
	Tropical	Cropland	Tillage	0–5	5.5	20	Silt Loam	2.99	Dry sieving	2–9	Sieving	<2	No Sand	21 %	25	28	1.53	0.42	53
	Temperate	Cropland	Tillage	0–30	7	20	Silt Loam	2.09	Wet sieving	>0.3	Sieving	<0.3	No Sand	28 %	25	20	1.11	0.10	11
García-Oliva et al., 2004	Tropical	Forest	Forest	0–5	6.9	5.2	Sandy Loam	3.34	Wet sieving	>0.25	Mortar crushed + sieving	<0.25	No Sand	N/A	25	22	0.65	–0.44	–35
Gijsman and Sanz, 1998	Tropical	Cropland	Tillage	0–7.5	5.3	5.2	Sandy Loam	5.69	Wet sieving	>2	Mortar crushed + sieving	<0.25	Sand	66 % WHC	24	41	1.18	0.16	17
	Tropical	Fallow land	Fallow	0–7.5	5.3	5.2	Sandy Loam	4.95	Wet sieving	>2	Mortar crushed + sieving	<0.25	Sand	66 % WHC	24	41	1.28	0.24	27
	Tropical	Cropland	Tillage	0–7.5	5.3	5.2	Sandy Loam	5.53	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	66 % WHC	24	41	1.09	0.09	9
Gupta and Germida, 1988 Jacobs et al., 2010	Tropical	Fallow land	Fallow	0–7.5	5.3	5.2	Sandy Loam	4.79	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	66 % WHC	24	41	1.13	0.12	13
	Temperate	Fallow	Fallow	0–15	6.5	10	Sandy Loam	2.95	Dry sieving	>0.25	Mortar crushed + sieving	<0.25	No Sand	N/A	25	14	1.10	0.09	10
	Temperate	Cropland	No Tillage	0–5	7	15.1	Silt Loam	1.25	Wet sieving	2–10	Mortar crushed + sieving	<0.25	No Sand	50 % WHC	22	28	1.20	0.13	14
Nyamadzawo et al., 2009	Temperate	Cropland	Tillage	0–5		15.1	Silt Loam	0.94	Wet sieving	2–10	Mortar crushed + sieving	<0.25	No Sand	50 % WHC	22	28	1.27	0.24	27
	Tropical	Fallow	Tillage	0–5	4.8	22	Sandy Loam	0.69	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	55 % WFPS	20	21	1.10	0.10	10
	Tropical	Fallow	No Tillage	0–5	4.8	22	Sandy Loam	0.80	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	55 % WFPS	20	21	1.15	0.13	14
Oorts et al., 2006	Tropical	Cropland	Tillage	0–5	4.8	22	Sandy Loam	0.59	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	55 % WFPS	20	21	1.06	0.06	6
	Tropical	Cropland	No Tillage	0–5	4.8	22	Sandy Loam	0.60	Wet sieving	0.25–2	Mortar crushed + sieving	<0.25	Sand	55 % WFPS	20	21	1.07	0.06	7
	Temperate	Cropland	Tillage	0–20	6.1	22.1	Silt Loam	1.10	Dry sieving	>12	Sieving	<0.05	No Sand	–63 KPa	15	40	2.03	0.71	103
	Temperate	Cropland	Tillage	0–20	6.1	21.6	Silt Loam	1.10	Dry sieving	>12	Sieving	<2	No Sand	–63 KPa	15	40	1.31	0.27	31

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Table 1 (continued)

Publications	Climatic information	Land use	Practices	Soil depth (cm)	pH	Clay content (%)	Soil texture	SOC (%)	Method of initial aggregate size separation	Initial aggregate size (mm)	Crushing operation	Crushed to (mm)	With inert sand or no sand	Moisture	Temperature (°C)	Time (days)	Carbon mineralization Crushed/Intact (RR)	Crushing effect (LnRR)	% Change in carbon Mineralization
	Temperate	Cropland	Tillage	0–20	6.1	21.6	Silt Loam	1.10	Dry sieving	>12	Sieving	<0.25	No Sand	–63 KPa	15	40	1.34	0.29	34
	Temperate	Cropland	No Tillage	5–20	6.1	20.8	Silt Loam	1.61	Dry sieving	>12	Sieving	<2	No Sand	–63 KPa	15	40	1.40	0.27	31
	Temperate	Cropland	No Tillage	5–20	6.1	20.8	Silt Loam	1.61	Dry sieving	>12	Sieving	<0.05	No Sand	–63 KPa	15	40	2.03	0.71	103
	Temperate	Cropland	No Tillage	0–5	4.8	18.8	Silt Loam	1.61	Dry sieving	>12	Sieving	<0.25	No Sand	–63 KPa	15	40	1.62	0.37	45
Powelson, 1980	Temperate	Fallow	Fallow	0–20	7.7	55	Clay	2.01	Dry sieving	<6.35	Mortar crushed + sieving	<1	No Sand	55 % WHC	25	20	0.24	0.22	25
Pulleman and Marinissen, 2004	Temperate	Cropland	Tillage	5–20	8.5	22	Loam	1.10	Dry sieving	>8	Mortar crushed + sieving	<0.05	No Sand	–10 KPa	20	35	2.01	0.70	101
	Temperate	Cropland	Tillage	0–10	8.6	22	Loam	1.10	Dry sieving	>8	Mortar crushed + sieving	<0.25	No Sand	–10 KPa	20	35	1.72	0.54	72
	Temperate	Grassland	Pasture	0–10	8.1	22	Loam	3.60	Dry sieving	>8	Mortar crushed + sieving	<0.05	No Sand	–10 KPa	20	35	2.10	0.74	109
Razafimbelo et al., 2006	Tropical	Cropland	Tillage	0–5	5.72	62	Clay	4.21	Wet sieving	<2	Mortar crushed + sieving	<0.2	Sand	80 % WHC	28	28	1.11	0.10	11
Razafimbelo et al., 2008	Tropical	Cropland	Tillage	0–5	5.72	62	Clay	3.21	Wet sieving	0.2–2	Mortar crushed + sieving	<0.05	Sand	80 % WHC	28	28	1.13	0.13	13
	Tropical	Cropland	No Tillage	0–5	5.72	62	Clay	4.61	Wet sieving	0.2–2	Mortar crushed + sieving	<0.05	Sand	80 % WHC	28	28	1.10	0.09	10
Razafimbelo et al., 2013	Tropical	Cropland	Tillage	0–5	–	59	Sandy Clay	2.41	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.14	0.13	14
	Tropical	Cropland	Tillage	0–5	–	59	Sandy Clay	1.28	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.15	0.14	15
	Tropical	Cropland	Tillage	0–5	–	20	Sandy Clay Loam	2.21	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.15	0.14	15
	Tropical	Cropland	Tillage	0–5	–	23	Sandy Clay Loam	1.16	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.09	0.08	8
	Tropical	Cropland	Tillage	0–5	–	12.5	Silt Loam	2.3	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.08	0.08	8
	Tropical	Cropland	Tillage	0–5	–	39	Sandy Clay	2.13	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.14	0.13	14
	Tropical	Cropland	Tillage	0–5	–	39	Sandy Clay	1.77	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.15	0.14	15
	Tropical	Cropland	Tillage	0–5	–	72.3	Clay	3.4	Dry sieving	>0.2		<0.2		–10 KPa	28	28	1.10	0.09	10

(continued on next page)

Table 1 (continued)

Publications	Climatic information	Land use	Practices	Soil depth (cm)	pH	Clay content (%)	Soil texture	SOC (%)	Method of initial aggregate size separation	Initial aggregate size (mm)	Crushing operation	Crushed to (mm)	With inert sand or no sand	Moisture	Temperature (°C)	Time (days)	Carbon mineralization Crushed/Intact (RR)	Crushing effect (LnRR)	% Change in carbon Mineralization
											Mortar crushed + sieving	<0.2	No Sand						
	Tropical	Cropland	Tillage	0–5	–	72.3	Clay	5.02	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.13	0.12	13
	Tropical	Cropland	Tillage	0–5	–	53.9	Sandy Clay	2.9	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.33	0.29	33
	Tropical	Cropland	Tillage	0–5	–	45.9	Sandy Clay	1.72	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.09	0.08	8
	Tropical	Cropland	Tillage	0–5	–	23.5	Sandy Clay Loam	1.42	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.07	0.07	7
	Tropical	Cropland	Tillage	0–5	–	25.7	Sandy Clay Loam	1.76	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.07	0.07	7
	Tropical	Cropland	Tillage	0–5	–	18.5	Silt Loam	0.73	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.02	0.02	2
	Tropical	Cropland	Tillage	0–5	–	27.3	Sandy Clay Loam	1.03	Dry sieving	>0.2	Mortar crushed + sieving	<0.2	No Sand	–10 KPa	28	28	1.02	0.02	2
Wang et al., 2014	Temperate	Cropland	Tillage	5–10	6.7	15	Silt Loam	0.67	Dry sieving	8–16	Mortar crushed + sieving	<0.125	No Sand	–10 KPa	20	52	1.28	0.23	26
Yang et al., 2020	Tropical	Grassland	Pasture	0–30	5	5.8	Sandy Loam	5.40	Dry sieving	0.25–2	Mortar crushed + sieving	<0.125	No Sand	–10 KPa	20	28	0.92	–0.08	–8
	Tropical	Grassland	Pasture	30–50	6.5	40.6	Silt Clay	2.14	Dry sieving	0.25–2	Mortar crushed + sieving	<0.125	No Sand	–10 KPa	20	28	1.03	0.03	3
	Tropical	Grassland	Pasture	0–30	6	19.5	Silt Loam	6.32	Dry sieving	0.25–2	Mortar crushed + sieving	<0.125	No Sand	–10 KPa	20	28	1.14	0.13	13
	Tropical	Grassland	Pasture	0–30	5	5.8	Sandy Loam	4.62	Dry sieving	>2	Mortar crushed + sieving	<0.125	No Sand	–10 KPa	20	28	1.19	0.17	18

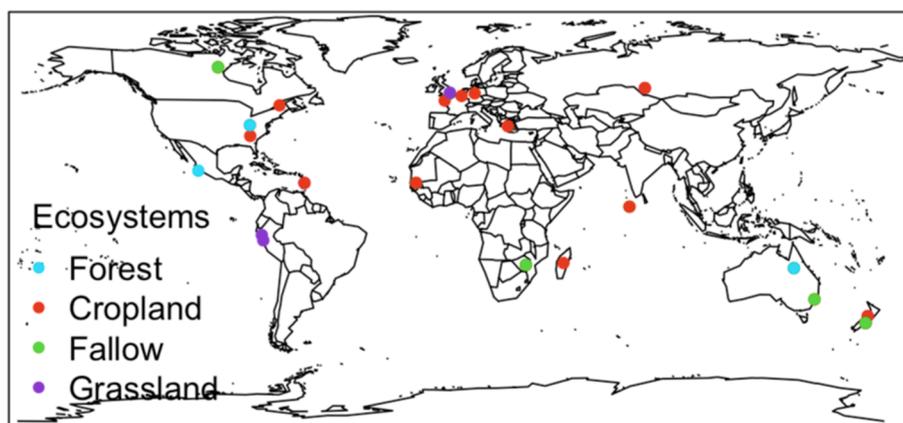


Fig. 2. Global distribution of the sites used in this meta-analysis. The world map was taken from a map database in R using packages ("maps" and "mapdata"). The points represent the geographical location of the sites based on the longitudes and latitudes collected in the studies.

2020). To investigate the relationships between soil characteristics and parameters of incubation experiments (clay content, carbon content, pH and incubation length) and the crushing effect (LnRR), we used linear regression models. To validate the significance tests of these models, we verified the residuals normality using the Shapiro-wilk test, the residuals homogeneity with the "residuals vs fitted plot" test, completed by a Breush-Pagan test and the residuals independence with the Durbin-Watson test. All statistical analyses were completed in R (version 4.0.2) and the "metafor" package used to random effect model (Viechtbauer, 2010, Harrer et al., 2019).

2.3. Publication bias

Kendall's rank correlation provides a distribution free test of independence and a measure of the strength of dependence between two variables. Kendall's rank correlation tests showed there were no significant relationships between the standardized crushing effect (LnRR) and their corresponding sample sizes ($p > 0.05$). Egger's test of the intercept (Egger et al., 1997) quantifies the funnel plot asymmetry and performs a statistical test. The function uses regression to test the relationship between the observed effect sizes and the standard error of the effect sizes. If this relationship is significant, that might indicate publication bias. However, asymmetry could have been caused by other reasons than publication bias. We see from the plot that in the case of our meta-analysis (see supplementary data Fig. S1), the plot is not asymmetrical. Eggers' test does not indicate the presence of funnel plot asymmetry ($p > 0.05$), which means that there is substantial symmetry in the Funnel plot. For all studies included in this meta-analysis, meta-estimation is not associated with significant publication bias.

3. Results

3.1. Data description

We found 22 studies, corresponding to 165 coupled observations that met our criteria. Some studies also reported crushing and incubation tests but were not included in this meta-analysis, because the incubation length was shorter than 2 weeks or the metric used did not allow to homogenize the data base. Experimental conditions, temperature, soil moisture, incubation length, and aggregate size differed among studies (Table 1). The size of the intact and crushed aggregates was systematically recorded and classified into: large aggregates (>10 mm), large macro-aggregates (2–8 mm), small macro-aggregates (0.25–2 mm) and micro-aggregates (<0.25 mm). The majority of the studies included in this meta-analysis were published between 1980 and 2009 and very few studies were conducted in the period 2010–2020. The studies included in this meta-analysis were conducted on a large variety of soils, climates

and ecosystems (Fig. 2, Table 1). About half of the data originated from tropical regions, 40 % from temperate regions and 5 % from Mediterranean and 5 % from boreal regions. Concerning land use patterns, we found more data in cropland (64 %) than in grassland (19 %) and fallow land (16 %). Unfortunately, very few experiments have been carried out on forest soils (1 %). In cropland, we distinguished between studies that compared conventional and no-tillage practices and those that did not (Table 1). Published studies mainly focused on topsoils (0–30 cm).

All studies performed soil incubations under controlled conditions in the laboratory with fixed temperature and soil moisture. The aggregates on which the crushing test was performed had been obtained either by dry sieving for 12 studies, or by wet sieving for 20 studies (see Table 1). To evaluate the amount of carbon protected within aggregates, the authors systematically monitored the carbon mineralization from the soil with the intact versus crushed aggregates. The crushing of the aggregates was not done under the same conditions in all studies. The crushing was done either by hand in a mortar or mechanically. After crushing, the soil was sieved through a smaller size sieve than the initial size of the aggregates. A large number of data focused on crushing large macro-aggregates ($n = 44$) and small macro-aggregates ($n = 85$). Very little data has been collected on large aggregates ($n = 28$) and micro aggregates ($n = 8$). The incubation temperatures ranged between 15 and 30 °C (Table 1). The soil moisture was expressed either as gravimetric water content, volumetric water content, percentage of water holding capacity (%WHC), percentage of water-filled pore space (%WFPS) or pressure units (Table 1). Unfortunately, these different units of soil moisture did not allow us to homogenize the data and thus to establish a relationship between soil moisture and the crushing effect (LnRR) on carbon mineralization. The incubation length ranged from a few days to more than one year. Crushed aggregates were incubated either with or without inert sand. Sand was used to enhance soil aeration and to prevent new aggregates formation. From the 22 studies included in this meta-analysis, only 2 studies reported pH values > 7 (i.e., Bischoff et al., 2017; Pulleman and Marinissen, 2004), which corresponded to only 6 soils out of 165. The higher pH was due to the presence of carbonate in these soils (Bischoff et al., 2017; Pulleman and Marinissen, 2004).

3.2. Crushing effect on cumulative carbon mineralization

The meta-estimation of the mean crushing effect on carbon mineralization showed a large heterogeneity between the studies ($I^2 > 75$ %). A high I^2 indicates that variability is caused by true heterogeneity among studies. The mean crushing effect of cumulative carbon mineralization was 0.27 (95 % CI from 0.22 to 0.32; $p < 0.0001$; Fig. 4c), suggesting that crushing aggregates stimulated significantly the carbon mineralization by + 31 % relative to intact aggregates (control). This extra mineralization following aggregate crushing represented on

average 0.83 % (95 % CI from 0.65 to 1.01 %; $p < 0.0001$; Table S2) of total SOC.

3.3. Experimental conditions affect the crushing effect on carbon mineralization

3.3.1. Incubation length

We have established a linear relationship between crushing effect and incubation length with all aggregate sizes and crushing intensities combined. The results reported in Fig. 3a, show a very scattered though significant negative correlation trend ($R^2 = 0,037$, $p = 0,023$). We also examined the correlation by data pair within the same study, when it was possible to obtain the cumulative mineralization over a short-term and long-term incubations. We observed the same tendencies as with the global data (results not presented). All means slopes measured between two dates in the same study were negative indicating that the crushing effect tends to decline with incubation length (Table 2). It was difficult to find data with identical incubation lengths in the articles obtained in our literature search. To reduce the possible effect of the incubation length on crushing effect (LnRR), we focused the meta-analysis on the data obtained with incubation lengths ranging from 2 weeks to one month. We chose this time range because most mineralization assays to determine the amount of carbon protected within aggregates were conducted at these time scales (e.g., Balesdent et al., 2000; Bossuyt et al., 2002; Chevallier et al., 2004, Chevallier et al., 2011).

3.3.2. Aggregates size and crushing intensity

We investigated first whether the wet or dry separation of the initial aggregates would affect the crushing effect, and compared similar initial aggregate sizes and crushing intensities. The results indicated a higher

crushing effect when the large macro-aggregates obtained by dry sieving were crushed into micro-aggregates (0.33 ± 0.11 CI 95 %) compared to the crushing of large macro-aggregates obtained by wet sieving (0.22 ± 0.13 CI 95 %). However, opposite results were obtained with small-macro-aggregates crushed into micro-aggregates (0.03 ± 0.05 CI 95 % and 0.13 ± 0.02 CI 95 % respectively for dry and wet sieving separation) (See Fig. S5). This result suggests that the effect of the initial aggregate separation method is not constant.

The crushing effect depended on the initial size of the aggregates. After crushing large aggregates (>10 mm), large macro-aggregates (2–8 mm), small macro-aggregates (0.25–2 mm) and micro-aggregates (<0.25 mm), the crushing effects were 0.50 (95 % CI from 0.37 to 0.63), 0.32 (95 % CI from 0.21 to 0.43), 0.13 (95 % CI from 0.10 to 0.15) and 0.43 (95 % CI from 0.15 to 0.71), respectively (Table 3 and Fig. 4b). Thus, aggregates crushing stimulated carbon mineralization relative to control, up to + 63 % in large aggregates, +38 % in large macro-aggregates, +14 % in small macro-aggregates and +54 % in micro-aggregates (Table 3).

Relative to large aggregates, the crushing intensity showed a significant difference between sieving sizes after crushing ($p < 0.0001$, Table 3a). The mean crushing effect on carbon mineralization when large aggregates (>10 mm) were reduced to sizes > 2 mm, <2 mm, <0.25 mm and < 0.05 mm were 0.34 (95 % CI from 0.17 to 0.51), 0.46 (95 % CI from -0.08 to 1.01), 0.48 (95 % CI from 0.31 to 0.65) and 0.83 (95 % CI from 0.09 to 1.58) respectively (Table 3a), suggesting that intense crushing allowed further deprotection and carbon mineralization. Similar results were obtained with large macro-aggregates (2–8 mm), where mean crushing effect on carbon mineralization after crushing them to > 2 mm, <2 mm, <0.25 mm and < 0.05 mm were 0.03 (95 % CI from -0.03 to 0.09), 0.48 (95 % CI from 0.06 to 0.89), 0.28 (95

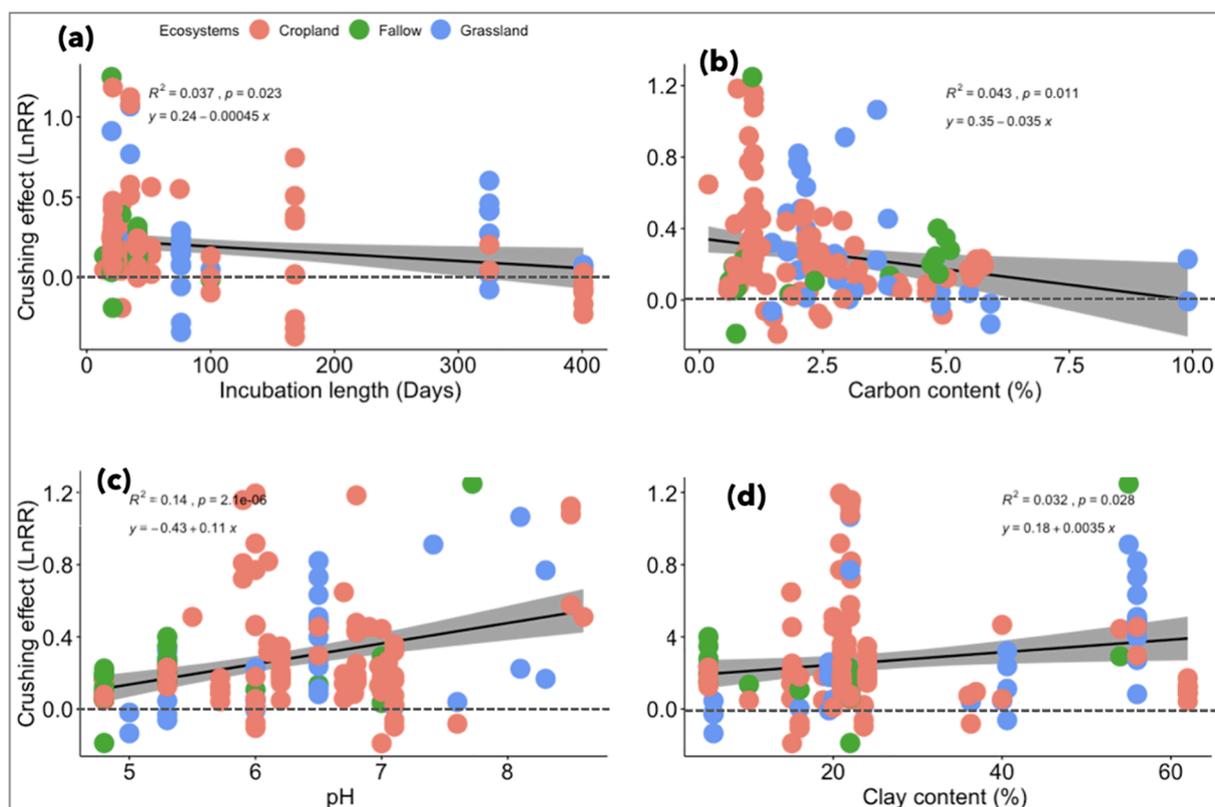


Fig. 3. Relationship between crushing effect (LnRR) of cumulative CO_2 emission and (a) incubation length; (b) Carbon content; (c) pH and (d) Clay content, by linear regression, all aggregate sizes combined. Each symbol represents an observation. The grey area represents the 95% confidence interval of the linear regression. A significant p -value (<0.05) indicates that part of the total heterogeneity can be explained by these variables. Dots below the dashed line correspond to a negative crushing effect ($\text{LnRR} < 0$) and above the dashed line correspond to a positive crushing effect ($\text{LnRR} > 0$). The red, green and blue dots represent cropland, fallow land and grassland respectively.

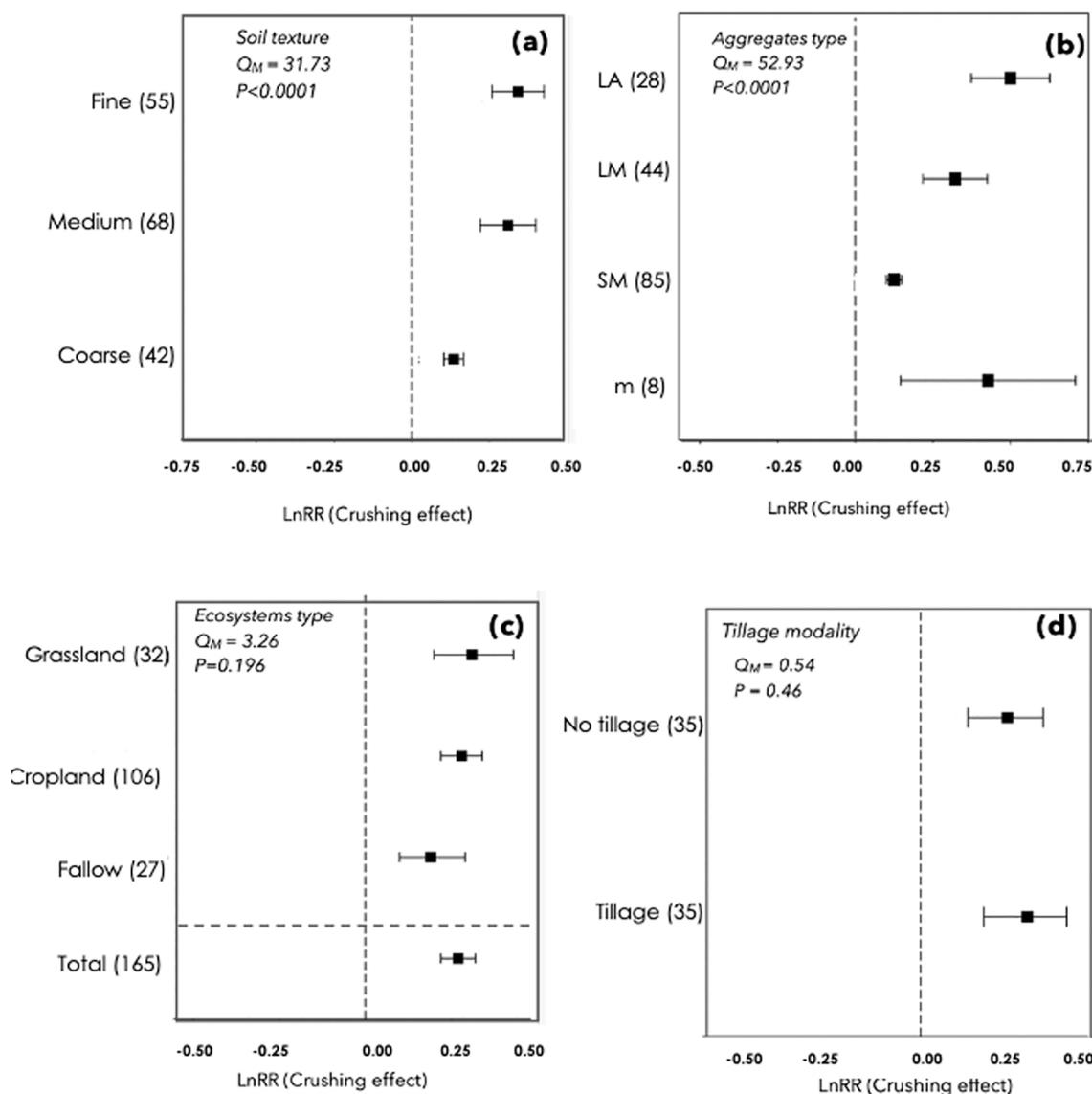


Fig. 4. Mean crushing effect (LnRR) of cumulative CO₂ emission (mean \pm 95% CI, CI is confidence interval) as affected by: (a) soil texture (Fine, Medium, Coarse); (b) aggregate type (LA: Large Aggregates, LM: Large Macro-aggregates, SM: Small Macro-aggregates, m: micro-aggregates); (c) Ecosystems (Cropland, Grassland, Fallow); (d) tillage modality (No-tillage vs Tillage). The sample sizes for each group are given on the left y-axis in brackets. The crushing effect is statistically significant if the 95% CI of the effect size does not overlap with the zero line ($P < 0.05$), and vice versa. LnRR > 0 represents a positive effect, while LnRR < 0 indicates a negative effect.

% CI from 0.20 to 0.36) and 0.85 (95 % CI from 0.38 to 1.31) respectively (Table 3b). However, it can be noted that the results were highly dispersed and sometimes the number of measurement points were low for some aggregate class sizes.

Overall, the studies that crushed soil to a size > 0.25 mm were considered to assess SOC protection within macroaggregates whereas studies that crushed soil to sizes much smaller than 0.25 mm were considered as assessments of microaggregate SOC protection (Six et al., 2002). The large aggregates (> 10 mm) crushed to > 0.25 mm diameters induced less % change in soil carbon mineralization (40–58 %) than the same aggregates crushed to < 0.25 mm diameter (62 %) and < 0.05 mm diameter (130 %) (Table 3a). The same tendency was observed for the large macro-aggregates (Table 3b), but the only difference was that the crushing effect was greater when these large macro-aggregates were crushed to < 2 mm than to < 0.25 mm. Crushing small macro-aggregates (0.25–2 mm) to a size < 0.25 mm had a smaller effect than crushing large macro-aggregates (2–8 mm) to < 0.25 mm, which also had a smaller effect than crushing large aggregates (> 10 mm) to < 0.25 mm

(Table 3). The same trends were observed when aggregates of different sizes were ground to < 0.05 mm.

3.4. Relationship between crushing effect and soil characteristics

3.4.1. Soil pH, carbon and clay content

We explored whether the variation in the crushing effect could be explained by soil characteristics, for all aggregates sizes and crushing intensities combined. There were significant correlations between the crushing effect and organic carbon content, clay content and soil pH ($p < 0.05$, Fig. 3): positive correlations with soil pH ($R^2 = 0.14$; $p = 0.023$) and clay content ($R^2 = 0.032$; $p = 0.028$), while soil organic carbon content ($R^2 = 0.043$; $p = 0.011$) was negatively correlated. However, each variable explained a very small proportion of the variance of the crushing effect. These relationships were similar for temperate and tropical soils with a large dispersion of data along the regression line (Supplementary data Fig. S2). Furthermore, we were unable to establish a relationship for Mediterranean and boreal soils because we had very

Table 2

Mean slopes of linear regression by data pair depending on incubation time. RR is the ratio of crushed aggregate cumulative mineralization relative to intact aggregates. Values are means \pm standard error.

Publications	Time1 (Days)	Time 2 (Days)	RR-Time1	RR-Time 2	Slope
Chevallier et al., 2004	21	325	1.62 \pm 0.33	1.28 \pm 0.28	-0.0013 \pm 0.0034
Yang et al., 2020	28	76	1.1 \pm 0.16	1.06 \pm 0.17	-0.0011 \pm 0.002
Bischoff et al., 2017	28	401	1.01 \pm 0.07	0.95 \pm 0.09	-0.00013 \pm 0.0002
Curtin et al., 2014	15	100	1.03 \pm 0.11	1.02 \pm 0.07	-0.00014 \pm 0.07
Curtin et al., 2014	14	68	1.03 \pm 0.03	0.98 \pm 0.04	-0.001 \pm 0.0002
Oorts et al., 2006	40	168	1.66 \pm 0.65	1.28 \pm 0.44	-0.0026 \pm 0.0021
D'Angelo et al., 2009	28	75	1.53 \pm 0.09	1.47 \pm 0.18	-0.0012 \pm 0.002

few data that came from the same study (Table 1).

We investigated the interaction of these different soil characteristics with the crushing effect. The interaction was only significant between soil pH and soil clay content ($p < 0.05$, see supplementary data Fig. S3 and Table S1).

3.4.2. Soil texture classes

Soil texture classes affected significantly the crushing effect on carbon mineralization ($p < 0.0001$, Fig. 4a). Coarse textured showed a lower crushing effect (0.13 ± 0.04 CI 95 %) than medium textured soils (0.31 ± 0.09 CI 95 %) and fine textured soils (0.34 ± 0.08 CI 95 %). Aggregate crushing stimulated + 40 %, +36 % and + 14 % carbon mineralization on fine, medium and coarse textured soils respectively.

3.5. Land use and tillage practices

Crushing effect (LnRR) on carbon mineralization was not significantly affected by land use ($p > 0.05$, Fig. 4c). The mean crushing effect

Table 3

Meta-estimates of the crushing effect according to initial aggregate size and crushing intensity: a) large aggregates (>10 mm), b) large macro-aggregates (2–8 mm), c) small macro-aggregates (0.25–2 mm) and d) micro-aggregates (0.25–0.05 mm). Sample size is indicated by 'N', meta-estimates of crushing effect (LnRR) are also reported as percentage change in carbon mineralization (% Change) with 95% confidence intervals (CI) (lower and upper). P-values are bolded when significant and the I^2 represents the level of heterogeneity between studies.

a) Large Aggregates (>10 mm)								
Variables	Size (mm)	N	LnRR	% Change	Low CI	Uper CI	I^2 (%)	P-value
Studies		26	0.50	65	0.37	0.62	98.2	<0.0001
	> 2	6	0.34	40	0.17	0.51	93.7	
	< 2	4	0.46	58	-0.08	1.01	99.7	
Crushed to	< 0.25	14	0.48	62	0.06	0.65	99.1	<0.0001
	< 0.05	4	0.83	130	0.09	1.58	99.7	
b) Large Macro-aggregates (2–8 mm)								
Variables	Size (mm)	N	LnRR	% Change	Low CI	Uper CI	I^2 (%)	P-value
Studies		43	0.32	38	0.22	0.43	99	<0.0001
	> 2	9	0.03	3	-0.03	0.09	94.1	
Crushed to	< 2	7	0.48	62	0.06	0.89	99.1	<0.0001
	< 0.25	23	0.28	32	0.20	0.36	99.0	
	< 0.05	5	0.85	133	0.38	1.32	98.8	
c) Small Macro-aggregates (0.25–2 mm)								
Variables	Size (mm)	N	LnRR	% Change	Low CI	Uper CI	I^2 (%)	P-value
Studies	< 0.25	85	0.13	14	0.10	0.15	87.8	<0.01
d) Micro-aggregates (<0.25 mm)								
Variables	Size (mm)	N	LnRR	% Change	Low CI	Uper CI	I^2 (%)	P-value
Crushed to	<0.05 mm	8	0.43	54	0.15	0.71	98.8	0.052

of cropland, grassland, fallow soil was 0.28 (95 % CI from 0.21 to 0.34), 0.31 (95 % CI from 0.19 to 0.43), 0.19 (95 % CI from 0.10 to 0.29) respectively (Fig. 4b). Crushing aggregates stimulated carbon mineralization of cropland, grassland and fallow land by + 32 \pm 7 %, +36 \pm 13 % and + 21 \pm 11 % respectively.

The meta-estimation of mean crushing effect on carbon mineralization relative to tillage practices, were 0.27 (95 % CI from 0.16 to 0.38) for tilled soils and 0.22 (95 % CI from 0.13 to 0.31) for no-till. Crushing aggregates stimulated carbon mineralization by + 30 \pm 11 % relative to intact aggregates in conventionally tilled soils, and + 25 \pm 9 % carbon mineralization in no-till soils. No-tillage did not induce significantly greater crushing effect in the aggregates than conventional tillage ($p > 0.05$; $Q_M = 0.54$; Fig. 4d).

4. Discussion

4.1. Crushing soil aggregates stimulates carbon mineralization

We observed that crushing aggregates increased cumulative carbon mineralization independent of their initial size. The differences between cumulative amount of CO_2 evolved from intact and crushed aggregates can be interpreted as the result of the mineralization of a previously protected pool of labile organic carbon (Hassink et al., 1993). This physically protected SOC represented an average of 31 % of total mineralized SOC after crushing (varying from 25 to 38 % of total mineralized SOC). Balesdent et al. (2000), earlier showed that the crushing macroaggregates to micro-aggregates (<0.15 \pm 0.30 mm) generally causes an increase in C and N mineralization in soil. However, some studies showed no such effect of crushing (Goebel et al., 2009; Plante et al., 2009). Plante et al. (2009) suspected that the disruption treatments used in their experiments (crushing of 2–4 mm aggregates to < 0.5 mm) was insufficient to release large amounts of physically protected soil organic carbon for decomposition, and that SOC was preferentially protected in micro-aggregates which were not destroyed in their treatment.

Our hypothesis that crushing aggregates stimulates carbon mineralization relative to intact aggregates was therefore confirmed.

However, it may be noted that after crushing, the mineralization is most often measured within a few weeks. Indeed, in this study, we presented results obtained between 2 and 4 weeks. Crushing tests cannot quantify all the organic carbon physically protected but only its very labile part. This physically protected SOC represented in average 0.83 % total SOC (95 % CI from 0.65 to 1.01 % SOC; $p < 0.0001$; Table S2) in the studies analyzed here. Physically protected SOC is usually estimated from fractionation of soil aggregates. For example, the organic carbon localized in microaggregates within stable macroaggregates represented in average 31 % of total SOC for three soils under different tillage regimes (Denef et al., 2004). The organic carbon localized within stable silt-size microaggregates represented 44 % of total SOC (Virto et al., 2008). The amount of protected SOC estimated by the crushing test is hence much less than the amount of SOC measured inside the aggregates by aggregate fractionation methods. The rather short incubations associated with crushing tests do not allow the mineralization of all the SOC situated inside aggregates.

4.2. Aggregates size and crushing intensity affect carbon mineralization

We hypothesized that both initial aggregate size and crushing intensity would affect the crushing effect. It is known that different aggregate sizes contain organic carbon with different residence times: the larger the aggregate size the smaller the residence time of carbon (Six and Jastrow, 2002). This may be due to both the differing nature and intrinsic decomposability of the organic carbon contained in aggregates of different sizes (large aggregates contain larger particulate organic matter, which is more decomposable) and to the extent of protection of SOC in the different aggregate size classes (both the extent of physical protection and protection by interaction with minerals being considered to be more important as aggregate sizes decrease). On average, crushing large aggregates (>10 mm) stimulated soil carbon mineralization by + 65 %, whereas crushing large macro-aggregates (2–8 mm), small macro-aggregates (0.25–2 mm) and micro-aggregates (<0.25 mm) stimulated soil carbon mineralization by + 38 %, +14 % and + 54 % respectively. We infer that large aggregates contained more labile and protected SOC than macro and micro-aggregates. It has been demonstrated, in a number of studies, that the larger the aggregates the younger is the organic matter it contains (Puget et al., 1995; Angers and Giroux, 1996; Six et al. 2006). Large aggregates compared to smaller aggregates could contain coarser POM. Crushing reduces the size of the POM included in the large aggregates (Chevallier et al. 2011). This reduction of POM size could increase their accessibility and decomposition (Angers and Recous, 1997). Crushing large aggregates, which contain both macro and micro-aggregates, to fine sizes could also deprotect the carbon in both macro and micro-aggregates.

However, crushing microaggregates tended to stimulate carbon mineralization more than crushing macroaggregates (large and small). The strong stimulation of carbon mineralization after micro-aggregates crushing could be explained by a high concentration of carbon and microorganisms in the microaggregates compared to macroaggregates (Rabbi et al. 2016). Crushing the micro-aggregates removes the physical barriers between the bacteria and the carbon substrates and also improves oxygenation in the micro-aggregates allowing decomposers to be more active (Young and Ritz, 2000). Angers and Recous (1997); Six and Jastrow (2002) and Besnard et al. (1996) demonstrated the stronger protection of organic carbon by micro-aggregates in comparison to macro-aggregates, that supports our results.

In this meta-analysis we also found that for defined initial aggregate size, the crushing effect depended on the final aggregate size after crushing, i.e. on the crushing intensity as shown for large aggregates (Table 3a) and large macro aggregates (Table 3b). In general, the destruction of aggregates to < 0.05 mm had a greater crushing effect than the destruction of aggregates to > 2 mm, <2 mm and < 0.25 mm regardless of the initial aggregate size (Table 3). These results confirm that macroaggregates (>0.25 mm) are less protective of organic carbon

than microaggregates (<0.25 mm). Some studies have shown that finely ground aggregates induced higher carbon mineralization (Powelson, 1980; Sørensen, 1983). There are some reasons that explain the effect of fine crushing on increasing carbon mineralization.

One hypothesis is that crushing the aggregates finely could lead to a physical breakdown of the particulate organic matter (POM) contained in the soil. This breakdown would increase the surface area of contact between the substrate, i.e. POM, and the soil microorganisms and then promote carbon mineralization during the incubation as shown by Angers and Recous (1997). This effect is especially important on large aggregates which probably contain more POM than macro and micro-aggregates.

The POM breakdown may not be the only factor explaining these results. Chevallier et al. (2011) have shown that adding plant residues increased the cumulative carbon mineralization, but grinding these plant residues to 200 μm did not increase the amounts of cumulative carbon mineralized. However, their study did not precisely assess the effect of residue grinding to finer sizes than 200 μm on carbon mineralization. Furthermore, the effect of reducing plant residues size probably depends on the residue quality, nitrogen availability and soil texture, as shown by Bossuyt et al. (2002).

Finally, the increase in carbon mineralization by finely crushing aggregates could also be explained by increased oxygen aeration (McLaren and Cameron, 1996).

4.3. Relationship between soil characteristics and crushing effect on carbon mineralization

The crushing effect was significantly but only weakly correlated to the investigated soil characteristics. It was positively correlated with the soil texture, the crushing effect being higher for fine and medium textured soils compared to coarse textured soils (Fig. 4a). Fine and medium textured soils are richer in clay (20–60 %) compared to coarse textured soils (2–5 %) (USDA). Some studies (Degens and Sparling, 1995; Goebel et al., 2009; Zhang et al., 2020) have suggested that clay-rich soils have a greater potential to release substrates after physical disturbance than coarser textured soils. Hassink (1992) observed that the increase in carbon and nitrogen mineralization after fine sieving was generally larger in loams and clays than in sandy soils which supports our results. Two reasons may explain that soils with fine texture physically protect more organic matter: the volume of fine-sized pores in which organic matter can be located and inaccessible to microorganisms or not well aerated increases with clay content (Hassink, 1992) and aggregate stability determining the life expectancy of protective sites. Indeed, sandy soils tend to have low aggregate stability compared with clayey ones, as sand particles interact much less with mineral and organic particles than clays (Bazzoffi et al., 1995).

Soil pH was weakly and positively correlated with the crushing effect (LnRR). A higher pH usually indicates the presence of Ca^{2+} ions on the exchange complex and above pH 7, possibly of CaCO_3 . It is widely accepted that Ca^{2+} and the presence of carbonates have a significant positive effect on aggregation and soil structural stability and therefore, indirectly influence the occlusion of SOC within stable aggregates and the persistence of these aggregates (Peterson, 1947; Martin et al., 1955; Bazzoffi et al., 1995; Fernández-Ugalde et al., 2014; Rowley et al., 2016). Other drivers influence aggregation and likely the SOC protection inside aggregates. For example, aggregation in tropical soils is mainly related to iron and aluminium oxides (Barthès et al., 2008). However, the information provided on soils in the collected articles did not allow to examine whether there was a correlation between iron and aluminium oxides and the crushing effect.

Carbon content was weakly and negatively linearly correlated with the crushing effect on carbon mineralization, but this correlation was significant only in temperate soils (Fig. S2). This result indicates that as soil organic carbon contents increase, the crushing effect is reduced. This result contradicts our hypothesis. Indeed, as high SOC content

promotes aggregation and aggregate stability, we expected that the crushing effect would increase with SOC content as observed for example by Razafimbelo et al. (2013) for macro-aggregates from Malagasy clay-rich tropical soils. Our finding may indicate that soils richer in SOC are in particular richer in free POM, i.e. a fraction that is not physically protected, or richer in mineral associated SOC, i.e. that is stabilized by another process than physical protection. (Cambardella and Elliot, 1992; Elliot et al., 1994; Bayer et al., 2001; Solvo et al., 2010).

Land use and tillage did not significantly affect carbon mineralization within aggregates.

Contrary to our hypothesis and previous results by Balesdent et al. (2000), the crushing effect was not significantly affected by the land use (Fig. 4c). Contrary to our expectations, the crushing effect was also not influenced by tillage practices (Fig. 4d). Indeed, we hypothesized the crushing effect to be higher in no-till soils that are considered to physically protect higher amounts of SOC compared to tilled soils, and Beare et al. (1994a) found that more C was released from a no-till soil upon a crushing test, compared to its tilled counterpart. There are a few possible explanations for this absence of effects: (i) only a limited number of studies compared tillage and no-tillage or (ii) the soil physico-chemical characteristics (carbonate content, clay content, Fe-Al minerals content) are factors that influence the process of aggregation more than land use, or (iii) the physical protection is removed during sample preparation (preparation by dry or wet sieving), or (iv) the additional SOC in no-till compared to tillage is not protected in the aggregates, but rather corresponds to free POM (Salvo et al., 2010), or (v) the additional SOC in no-till compared to tilled or in forest and grassland compared to cropland can only mineralize very slowly even if freed from aggregates because of other stabilization processes, so that the duration of the incubations is too short to target it, as previously mentioned.

5. Conclusion

While crushing tests have been used for decades to demonstrate or quantify the occurrence of physical protection in soils, no synthesis was available on their outputs. Through this meta-analysis, we evaluated the impact of aggregate crushing on carbon mineralization in incubation experiments. We found that, on average, soil aggregates crushing increased soil carbon mineralization compared to intact aggregates. This confirmed the role of soil structure in protecting SOC from biodegradation, since carbon mineralization was enhanced when soil structure is disrupted, and confirmed that physical protection is more important at fine scales of soil organization, i.e. within microaggregates. Crushing tests are commonly used to quantify the importance of physical protection of soil organic matter. Indeed, it is relatively easy to implement in the laboratory, and our study confirms that the results of crushing tests are influenced by expected drivers of physical protection (soil texture, pH), which sustains their use. However, contrary to our expectations that more organic carbon would be being physically protected in situations associated with little soil disturbance (no-till, permanent grasslands and forest) than in situations with soil disturbance, our meta-analysis revealed no difference. This lack of difference remains to be explained. Furthermore, as most of crushing tests are associated with short incubations, crushing tests are limited to quantify physically protected labile organic carbon, i.e. decomposable in a few weeks. Organic carbon that has longer residence times because of other processes will not be detected. Even if this simple crushing method was successfully used to estimate physically protected SOC, this meta-analysis showed that the size of the intact and crushed aggregates varied considerably across studies, as well as incubation conditions. These diverse experimental protocols led to contrasting results and indicate the need to standardize the aggregate destruction and incubation procedure (aggregate size, moisture, incubation length etc.) in order to be able to compare results and to understand the drivers of SOC physical protection better.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2022.116089>.

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