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### Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and associated organic carbon fractions in conservation agriculture: A review

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### Abstract

Decomposition of fresh crop residues quickly leads to the formation of water-stable (WS) soil macroaggregates. How the initial soil organic carbon (SOC) concentration affects macro-aggregation is still unclear, and the consequences for short-term retention of crop-residue C and N are unknown. Waterstable macro-aggregate formation per unit of residue-C added was greater in the SOC-poor subsoil than in the SOC-rich topsoil. Large macro-aggregates (>1000 mm) in the SOC-poor subsoil were enriched in <sup>13</sup>C and <sup>15</sup>N in both particulate organic matter (POM, >50 mm) and fine particle size (<50 mm) fractions compared to the SOC-rich topsoil. We postulate that the retention of residue-C and -N in both POM and fine fractions within WS macro-aggregates is due to the large-scale occlusion of coarse material and small-scale adsorption of organic substances occurring concomitantly in soil. Although the labile pools of SOC were positively affected by conservation tillage practices (CT-NT, NT-NT, and NT-CT), the less labile pool was only influenced by the continuous NT and NT-CT in the 0- to 5-cm depth. Plots under NT-NT and NT-CT had about 27 and 19% higher labile SOC pool than CT-CT plots (5.65 g C kg<sup>-1</sup> soil), and NT-NT and NT-CT plots had about 14 and 11% higher less labile SOC pool than CT-CT plots (2.61 g C kg<sup>-1</sup> soil) in that soil layer. SOC storage decreased with soil depth, with a significant accumulation at 0-20cm depth. Across treatments, aggregate-associated C at a depth of0-10cm was higher in the conservation agriculture (CA) treatment than in the conventional tillage (CT) treatment. The advantage of the CA treatment weakened with soil depth, while the amount of aggregate-associated C remained higher for the CT treatment. There were more macro-aggregates in the CA treatment than in the CT treatment, while CT treatment had more micro-aggregates. The sum of macro-aggregate contributing rates for clay-humus stability of soil organic C (SOC) was significantly superior to that of the microaggregates. Water-stable aggregates increased by 34.5% in the CA with residue retention treatment, effectively improving the soil structure. Furthermore, 0.25–1.00 and 1–2mm aggregates had the highest SOC microbial biomass storage and responded rapidly to the various tillage treatments. Greater proportion of micro-aggregates within macro-aggregates in the plots under NT-NT compared with CT-CT was also observed in the surface layer only. Plots under NT-NT had about 10% higher coarse (250-2000 µm) intra-aggregate particulate organic matter-C (iPOM-C) within >2000 µm sand free aggregates in the 0- to 5-cm soil layer compared with CT-CT plots. The fine (53-250 µm) iPOM-C within the 250to 2000-µm aggregates was also higher in the continuous NT plots compared with CT within both >2000 and 250 to 2000 µm sand free aggregate size classes in that soil layer. The application of conservation tillage practices to be crucial for maintaining soil quality and stability of organic carbon in soils of the North West IGP.

Keywords: Macro-aggregation, SOC stability, density fractionation, soil aggregate, soil depth

### Introduction

The size of crop residues may critically affect the contact area between organic materials and soil minerals and the sub-sequentent formation of stabilized SOC (Iqbal *et al.*, 2014)<sup>[8]</sup>. On one hand, fine-sized residue particles can expose more surface area for microbes and is thus conducive to microbial processing of residue C. Consequently, microbes will invest less C into substrate acquisition, and then enhance the ratio of residue C allocated to microbial biomass and dissolved organic C (DOC) (Cotrufo *et al.*, 2013; Spohn *et al.*, 2016)<sup>[3, 28]</sup>. Because microbial biomass and DOC produced during microbial decomposition of plant residues constitute a major component that interacts with soil minerals (Miltner *et al.* 2012; Cotrufo

*et al.*, 2015) <sup>[14, 4]</sup>, higher microbial activities and growth may increase DOC and microbial biomass, and thus facilitate soil C retention. On the other hand, fine-sized residues often decompose faster than the coarse-sized ones, particularly in sandy and sandy loam soils (Sims and Frederick, 1970) <sup>[23]</sup>

suggesting that soils with low content of clay minerals may limit the accumulation of residue-derived Cin soil and the ultimate fate of the decomposition products may be strongly dependent on soil texture (Cotrufo *et al.*, 2013)<sup>[3]</sup>.



Soil microorganisms strongly affect processing of organic carbon(C) in soil (Schmidt et al., 2011)<sup>[20]</sup>. The ratio of organic C allocated to growth over organic C taken up by the microbial community composition is termed microbial C use efficiency (CUE), and is an important synthetic representation of the microbial community metabolism (Sinsabaugh et al., 2013) <sup>[24]</sup>. Despiteits importance for C cycling, it is still not well understood, which factors shape microbial CUE. The soil microbial biomass has a relatively well constrained C:N: P ratio, ranging between 60:7:1 and 42:6:1 on a global average(Xu et al., 2013)<sup>[31]</sup>. When feeding on substrate with a stoichiometry unfavourable for the maintenance of the microbial biomass stoichiometry, microorganisms have to allocate more energy into the acquisition of missing elements, for example by exoenzymes, in order to be able to maintain their biomass. Thus, microorganisms feeding on substrate with an unfavourable stoichiometry likely partition more C taken up to respiration than microorganisms feeding on substrate that matches their nutritional requirements, which leads to a decrease in CUE (Sinsabaugh et al., 2013)<sup>[24]</sup>.

Density fractionation, which separates organic debris residing inside and outside aggregates (light fraction, LF) from OM bound to minerals (heavy fraction, HF), provides one method to study physical or physicochemical stabilization mechanisms (Sohi *et al.*, 2005) <sup>[15]</sup>. Organic carbon in the LF basically consists of plant and animal residues at different stages of decomposition. A common concept assumes that LF-OC released from soil upon disruption of aggregates (occluded light fraction, Olf) has a smaller particle size, is chemically more degraded, and older than the physically unprotected free LF (fLF). Organic matter of the HF comprises larger contributions of microbial residues than LF-OC (Kogel-Knabner *et al.*, 2008). Compounds forming

HF-OC can be direct decomposition products of LF-OC (Sanaullah *et al.*, 2011), or dissolved OC (DOC) from the percolating soil solution (Kalbitz and Kaiser, 2008). Organic carbon storage in the HF is controlled by the availability and nature of binding sites at mineral surfaces. Iron (Fe) and Alhydrous oxides rather than other clay-sized particles were shown to be good predictors for HF-OC, especially in acid forest soils. The few studies of density fractions over the entire soil profile show declining contributions of LF-OC to total OC with soil depth. Strongly increasing carbon ages, especially of HF-OC, with soil depth were assumed to be due to greater stability of HF-OC at depth and can reflect differences in physicochemical protection between topsoil and subsoil layers.

Soil physical fractionation techniques can improve our ability to detect management-induced changes in pools of C and N, and have been used widely in agricultural ecosystems (Powlson *et al.*, 2012) <sup>[17]</sup>. Fractionation provides an indication of the physical location of C and Nin the soil matrix (Sollins *et al.*, 1996) <sup>[27]</sup>. Turnover of SOM is governed, in part, by the accessibility of organic substrates to decomposers (Dungait *et al.*, 2012) <sup>[5]</sup>, and also by the chemical quality of the material. Assessments of SOM chemical characteristics are commonly used to infer its potential reactivity. By combining physical fractionation and chemical characterization, it is possible to identifyfractions with different SOM stabilization potentials (Poirier *et al.*, 2005) <sup>[15]</sup> to assess their relevance for long-termsoil C and N storage.

Previous studies have shown that straw residue and organic fertilizer are stored primarily in the free particulate organic matter (POM) fraction by forming intra-micro-aggregate POM within macro-aggregates (im MPOM) (Jagadamma & Lal, 2010) <sup>[9]</sup>. The im MPOM, which has been consistently considered an important agent to promote the binding of micro-aggregates into macro-aggregates, enhances the physical protection of organic C (Six et al., 1998) <sup>[26]</sup>. The application of chemical N was reported to increase the organic C contents in macro-aggregates and in free silt and clay fractions in a sandy loam soil (Yu et al., 2012a) [32]. Inorganic fertilizer can also decrease the SOC content in the silt + clay fraction, as reported for a 20-year field experiment. and when incorporated in combination with straw it can increase SOC (Li et al., 2013). However, the effect of different rates of N addition and straw management on SOC accumulation in whole soil is not well understood. The objectives of the present review study were as follows: (i) to quantify the effects of N management and straw return on SOC stocks and storage and stability of organic carbon in the soil profile, (ii) to evaluate the effects of conservation agriculture (CA) strategy and straw return on depth, occlusion within aggregates associated C and (iii) to propose reasonable CA and straw management strategies to improve the associated organic carbon fractions in intensively managed soil on the North West IGP.

Reported that the distribution of SOC across the four different aggregate size classes in all six soil types [Fig.1a] the total amount of SOC declines with depth in all soils; the proportion of SOC associated with large and small macro-aggregates declines with depth in all soils and a larger proportion of the SOC is associated with micro-aggregates and silt plus clay fractions in soils affected by clay illuviation (namely: Typical Luvisols (TLu), Stagnic Luvisols (SLu) and Typical Surfacewater Gleys (TSWG)) than in Brown Earths (Humic Brown Earths (HBE), Typical Brown Earths (TBE) and Stagnic Brown Earths (SBE)).As a result, most (68.9% • } 11.5) of the SOC was found within the top 30 cm of the soil profiles, and a significant proportion (84% } 9.5) of this topsoil SOC was located within large and small macro-aggregates, as indicated by the predominance of the 'large bubbles' in quadrant B. Macro-aggregates are formed around particulate organic matter (POM), which is derived from the decomposition of fresh residues(Six *et al.*, 2000) <sup>[25]</sup>. We suggest that within the topsoil (0–30 cm), the process of aggregate formation is primarily influenced by vegetation type and root exudates, which is in line with other studies suggesting that topsoil SOC characteristics mainly reflects the vegetation type (Rumpel and Knabner, 2011; Mikutta *et al.*, 2009) <sup>[18, 13]</sup>.

Schrumpf *et al.* (2013) <sup>[21]</sup> revealed that the amount of OC stored in density fractions and their depth distribution differed between land use types. Croplands had smallest average LF-OC stocks in full soil profiles ( $0.85\pm0.41 \text{ kgOCm}^{-2}$  in 0–60 cm, because large amounts of fossil OC contributed to LF-OC in sub-soils) of all studied land use types [Fig. 1b]. Moreover, evolution of CO<sub>2</sub> per g soil showed large variation among sites and decreased with soil depth from a mean of  $13.7\pm8.4 \mu$ gCO<sub>2</sub>-C (g dwt)<sup>-1</sup> day<sup>-1</sup> in the 0–5 cm layer to  $1.5\pm0.7 \mu$ gCO<sub>2</sub>-C (g dwt)<sup>-1</sup> day<sup>-1</sup> in the 20–30 cm layer [Fig.1c]. Similar to mineralization rates (CO<sub>2</sub>-release per g soil), specific mineralization rates (CO<sub>2</sub>-release per gOC in the samples) also declined consistently with soil depth indicating reduced OC turnover at depth [Fig.1c].



Fig 1: (a) Relative distribution of SOC within aggregates by depth [Source: Sallan *et al.*, 2017],
Fig 1(b): Soil OC stocks of the litter layer and in density fractions[Source: Schrumpf *et al.*, 2013]<sup>[21]</sup>,
Fig 1(c): Average C mineralization in the mineral soil[Source: Schrumpf *et al.*, 2013]<sup>[21]</sup>

Mikha *et al.* (2012) <sup>[12]</sup> observed that on a fixed-depth basis, NT and RT had 21% more SOC, at the 0- to 30-cm depth than CT and MP. However, on equivalent mass basis (ESM), SOC was greater with NT, MP, and RT by 11% compared with CT. Conservation practices, NT and RT, had more macroaggregation and consequently greater soil stability compared with CT and MP. Tillage practices significantly impacted whole SOC distribution between POM-C and mineral-associated organic matter C (MAOM-C). The POM-C vs. MAOM-Component of the whole SOC was 23 vs. 77% at 0 to 5 cm and 10 vs. 90% at 5- to 20-cm depth. The POM-C associated with NT and RT, accounted for17% of SOC where POM-C accounted for 12% of SOC with CT and MP at 0-to 20-cm depth. Redistribution and stratification of SOC, POM,

and POM-Cwere observed especially with MP [Fig.2a &2b]. Zhao *et al.* (2014) <sup>[33]</sup> Results showed that soil aggregate fractions (> 0.25 mm) of four vegetation types were significantly (P < 0.05) higher in 40–60 cm soil depth under Po, Pt, and Ab compared with Sc and the SOC distribution in macro-aggregates (> 0.25 mm) under Rr, Po, Pt, and Ab was higher more than 37.7, 92.4, 92.5, 79.1%, respectively in 40–60 cm compared with Sc [Fig. 2c]. Huang *et al.* (2010) <sup>[7]</sup>, which suggested that macro-aggregates (> 0.25 mm) fractions from severely eroded *Ultisols* decreased with the increase of particle sizes compared with slightly and moderately eroded *Ultisols*. Zheng *et al.* (2011a) <sup>[35]</sup> suggested that soil aggregate with size of > 0.25 mmwas higher after land use change, and that in the forest soil it was higher than in slope farmland soil.



Fig 2(a): Sand-free water stable aggregates (g aggregates kg<sup>-1</sup> soil) atthe 0- to 5-cm, 5- to 10-cm, and 10- to 20-cm depths as affected by conventional tillage (CT), moldboard plow (MP), no-tillage (NT), andreduced tillage (RT) [Source: Mikha *et al.*, 2012]<sup>[12]</sup>,
 Fig 2(b): Total particulate organic matter content (g POM kg<sup>-1</sup> soil), at the 0- to 5-cm, 5- to10-cm, and 10- to 20-cm depth as affected by conventional tillage (CT), moldboard plow (MP), no-tillage (NT), and reduced tillage (RT)[Source: Mikha *et al.*, 2012]<sup>[12]</sup>,
 Fig (2c): Distribution of soil aggregate fractions of different vegetation types [Source: Zhao *et al.*, 2014]<sup>[33]</sup>

Schrumpf *et al.* (2013) <sup>[21]</sup> also found that the density fractionation separates total soil OC into fractions of different OC-to-TN ratios and HF-OC was in a more advanced decomposition stage than LF-OC, and that more microbial derived OC contributed to HF-OC. This is in line with smaller OC-to-TN-ratios [Fig.3a] Zhao *et al.* (2014) <sup>[33]</sup> also found that the micro-aggregates (< 0.25 mm) of five vegetation types had higher SOC content than macro-aggregates. However, SOC contents in macro-aggregates (> 0.25 mm) of

Rr, Po, Pt, and Ab were greater than that of Sc, suggesting that the distribution of SOC contents appeared to have shifted from the micro-aggregates (< 0.25 mm) in low C input systems to the macro-aggregates in high C input systems. It was mainly due to micro-aggregate formation within macro-aggregates. Micro-aggregates occluded in the macro-aggregates can serve as an indicator for C sequestration [Fig.3b].



Fig 3(a): characteristics of density fractions in topsoil and subsoil layers and their relation to soil respiration (fLF: free light fraction, oLF: occluded light fraction, HF: heavy fraction, OC: OC concentration)[Source: Schrumpf *et al.*, 2013]<sup>[21]</sup>
 Fig 3(b): Distribution of soil organic carbon of different vegetation types [Source: Zhao *et al.*, 2014]<sup>[33]</sup>

Ryals et al. (2014) [19] reported that Soil C and N content of surface (0-10 cm) soils were greater in amended versus control plots by the end of year one and remained elevated by the end of year three at the valley grassland [Fig.4a]. At the end of year three, the total soil C content of the valley site was  $53 \pm 2$  Mg C/ha and  $63 \pm 5$  Mg C/ha in 0-50 cm depth for control and amended plots, respectively. Total N in 0-50 cm depth was 3.17  $\pm$  0.14 Mg N/ha for the control and 3.73  $\pm$ 0.23 Mg N/ha for the amended plots. The positive effect on soil C content was greatest in the first year following amendment application. The positive effect on N content was sustained through all three years following amendment [Fig. 4b]. However, organic matter amendments significantly increased the C content of the FLF by  $2.56 \pm 1.13$  Mg C/ha and  $1.82 \pm 0.99$  Mg C/ha in the top 10 cm of soil at valley and coastal grasslands, respectively [Fig. 4b]). The C content of the OLF at the coastal grassland increased by  $1.29 \pm 1.75$  Mg C/ha with a significant blocks effect and the OLF C content at the valley grassland showed a similar but non-significant trend. The HFC content did not vary significantly by treatment. Nitrogen concentrations and content increased significantly in all soil fractions at the valley grassland and in the FLF and

### OLF at the coastal grassland [Fig.4b].

Shen et al. (2018)<sup>[22]</sup> revealed thatSOC, EOC, DOC, and MBC decreased with increasing soil depth, and significant difference were only observed in the SOC and DOC contents among the three soil layers. The EOC content in the 0–10cm layer was significantly higher than that in the other soil layers. In the 0-10 cm soil layer, SOC content under the LIT and HIT treatment were significantly lower than those in the CK and SC plots, but in 0-30 cm soil layer, significant difference between the HIT and CK treatment was only observed. The contents of DOC under the SC and HIT treatment were significantly higher than those in CK and LIT treatment in 0-10 cm soil layer and the corresponding values in the 10-20 cm layer were significantly higher than those in the CK. The content of EOC in the 0–10 cm layer was significantly higher in the HIT treatment than that in other three treated plots. In addition, the content of MBC in the three treated plots washigher than those in the CK, and, in particular, there was only a significant difference between LIT and CK treatment observed in three soil layers [Fig.4c].Similar results were found by (Chen et al., 2016; Achat et al., 2015) [2, 1].



Fig 4(a): Total soil organic C and total N content to 10 cm depth prior to and three years following organic matter amendment at two grassland types [Source: Ryals *et al.*, 2014] <sup>[19]</sup>

Fig 4(b):The contribution of three soil fractions to total soil organic C content from 0 to 10 cm depth for control and amended soils in two grassland ecosystems [Source: Ryals *et al.*, 2014]<sup>[19]</sup>

Fig (4c): Soil LOC fractions in the four forest management treatments [Source:Shenet al., 2018]<sup>[22]</sup>

Zibilsk *et al.* (2002) <sup>[36]</sup> reported that the No-till resulted in significantly greater soil organic C in the top 4 cm of soil, where the organic C concentration was 58% greater than in the top 4 cm of the plow-till treatment. In the 4–8 cm depth, organic C was 15% greater than the plow-till control [Fig.5a]. The differences were relatively modest, but consistent with organic C gains observed in hot climates where conservation tillage has been adopted. Higher concentrations of total soil N

occurred in the same treatments; however a significant reduction in N was detected below 12 cm in the ridge-till treatment [Fig.5b]. The relatively low amount of readily oxidizable C (ROC) in all tillage treatments suggests that much of the soil organic C gained is humic in nature which would be expected to improve C sequestration in this soil [Fig.5c].



Fig. 5(a): Soil organic carbon by depth after 9 years of no-till, ridge-till or plow-till treatment [Source: Zibilsket al., 2002] <sup>[36]</sup>
 Fig 5(b): Total soil nitrogen by depth after 9 years of no-till, ridge-till or plow-till treatment [Source: Zibilsket al., 2002] <sup>[36]</sup>
 Fig 5(c): Readily oxidizable soil carbon by depth after 9 years of no-till, ridge-till or plow-till treatment [Source: Zibilsket al., 2002] <sup>[36]</sup>

Ye *et al.* (2017) observed that the microbial respiration rate of samples from the two sized litter treatments showed similar temporal pattern. Increasing litter addition rate resulted in markedly higher microbial respiration rate [Fig.6a]. However, different residue sizes did not significantly affect the microbial respiration rate. Also, increasing litter addition rate made no difference when the respiration rate was expressed per unit. The cumulative C lost by microbial respiration significantly increased with the high residue rate,while residue size did not affect the cumulative C loss [Fig.6a]. When normalized by soil C, cumulative C effluxshowed a trend of convergence among the treatments with different residue addition rates [Fig.6a].

Both residue addition rates and sizes significantly affected DOC, DIN, MBC and MBN [Fig.6b]. DOC increased along the residue addition rates and decreased with the residue size across the whole incubation period, leading to a significant addition rate  $\times$  residue size interaction [Fig.6b]. In general, the differences for DIN, MBC and MBN were significant only

at 60 days [Fig.6b]. Miltner et al. (2012) <sup>[14]</sup> also found that reducing residue size increases microbial C use efficiency. Moreover, microbial biomass is a major contributor to stable SOC. These results suggest that over time, the MBC and MBC-derived C under the fine-sized residue treatment may constitute a significant source of stable SOC through strong physical and chemical bonding to the mineral soil matrix (Cotrufo et al., 2015)<sup>[4]</sup>. Second, fine-sized residues may contribute more to the DOC pool probably due to more efficient leaching and microbial decay of residues, as evidenced by more DOC in soil amended withfine-sized residues compared with the coarse-sized ones Higher residue addition rates resulted in higher bulk soil C, organic C in the heavy soil fraction and Fe/Al-associated C [Fig. 6c]. Although residue size had no significant impacts on bulk soil C, fine-sized residues led to more C accumulationin the heavy soil fraction as well as Fe/Al-bound C than coarse-sized residue [Fig. 6c].



Fig. 6(a): Effects of residue addition rate and size on microbial respiration rate (a), C-normalized respirationrate (b), cumulative C efflux (c) and C-normalized cumulative C efflux (d) [Source: Ye *et al.*, 2017]

Fig. 6(b):Effects of residue addition rate and size on dissolved organic C (a), dissolved inorganic N (b),microbial biomass C (c) and microbial biomass N (d) [Source: Ye *et al.*, 2017]

Fig. 6(c):Effects of residue addition rate and size on bulk soil C (a), total C (b) and Fe/Al-bound C (c) in heavyfraction [Source:Ye et al., 2017]

Gu *et al.* (2016) <sup>[6]</sup> revealed that SOC concentration in all treatments decreased with soil depth. The significant differences of SOC among treatments were solely at depths of 0-40 cm, where soil physicochemical properties changed. Further changes would have occurred following activity by

microorganisms. Average SOC content at depths of 0-40cm in ST and GT were 6.26 g kg<sup>-1</sup> and 6.59 gKg<sup>-1</sup> respectively, significantly higher than that of 5.44 g kg<sup>-1</sup> in CK [Fig 7a.]. The use of ST and GT increased SOC by 15.15% and 21.14% respectively. In the course of the growing season, SOC

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concentrations in all treatments presented substantial changes with seasons. The maximum SOC was recorded in the dry and cold season, and the minimum in the warm and wet season. Gu *et al.* (2016) <sup>[6]</sup> also found that compared to the control

without cover (CK), ST and GT treatments increased the control without cover (CK), ST and GT treatments increased the contents of SOC,LOC, DOC, POC and EOC by 14.73%, 16.5%, 22.5%, 41.5% and 21%, respectively, in the 0-40 cm soil layer, and by 17%, 14%, 19%, and 30%, respectively, in the 0-100 cm soil layer [Fig.7b]. The contents of organic carbon and its active fractions decreased with increasing soil depth in all of the treatments. SOC was accumulated in the period of December to the following March. The contents of soil DOC and LOC were high in January to March, while the contents of soil POC and EOC were high in June to September. The relative contents of soil organic carbon fractions were POC > EOC > LOC > DOC over the four years. However, ST and GT treatments significantly increased

soil DOC concentrations at depths of 0-40 cm, by 28.56% and 23.33% respectively, [Fig.7c] compared to CK, but there was no difference between ST and GT treatments at each layer of the soil profile. The increase in DOC with ST may be due to the soluble decomposed organic materials of the straw, while the increase in DOC with GT could possibly be attributed to an increase in organic acids and water-soluble carbohydrates from rhizo-deposition and root exudates. In addition, a decrease in surface runoff under GT and ST was an important reason for the increased DOC, as DOC may be lost with runoff. Compared with CK, the DOC in GT and ST was favorably leached, deposited and absorbed into the subsoil layer, resulting in higher concentrations of DOC at depths of 20-40 cm [Fig.7c]. This was probably because of low soil bulk density in ST, and in GT lower pH would have increased DOC adsorption by soil (Jardine et al., 1989]<sup>[10]</sup>.



Fig 7(a): Changes of soil total organic carbon [Source: Gu *et al.*, 2016] <sup>[6]</sup>
Fig 7(b): Dynamic changes of carbon fractions [Source: Gu *et al.*, 2016] <sup>[6]</sup>
Fig 7(c): Content of Carbon fractions at different depths [Source: Gu *et al.*, 2016] <sup>[6]</sup>

Tian et al. (2018) <sup>[29, 34]</sup> reported that theincreased organic C and totalN concentrations in each size fraction [Fig.8a]. Organic Concentrations in the <0.053-, 0.053- to 0.25-, 0.25to 2.0-, and >2.0-mm fractions were 14.0, 12.0, 14.4, 24.1% greater, respectively, in OF than in CF. Similarly, OF increased total Nconcentrations in the <0.053-, 0.053- to 0.25-, 0.25-2.0, and>2.0-mm fractions by 11.7, 9.4, 9.5, 17.0%, respectively. Organic farming also significantly increased the organic C and total N concentrations of micro-aggregates occluded with in macro-aggregates [Fig.8a]. Organic C concentrations in the<0.053 and 0.053 to 0.25 microaggregates were 14.0 and 13.8% greater, respectively, in OF than in CF. The total N concentrations in the <0.053 and 0.053 to 0.25 micro-aggregates were23.1 and 19.4% greater, respectively, in OF than in CF. Moreover, the combining the four aggregate size classes, the total amount of soil organic C storage was 20% greater in OF (5.60 kg m<sup>-2</sup>)than in CF (4.67 kg m<sup>-2</sup>) [Fig.8b]. Total N storage was 18.5% greater in OF  $(0.58 \text{ kg m}^{-2})$  than in CF  $(0.46 \text{ kg m}^{-2})$  [Fig.8b].In both treatments, organic C and total N storage were greatest in the <0.053-mm fraction and least in the >2-mm fraction. Organic farming significantly increased organic C storage in the 0.053 to 0.25 and >2.0 fractions, but had no significant effect on the <0.053-mm and 0.25 to 2.0 fractions. In comparison, organic farming significantly increased total N storage in all except the<0.053-mm fraction.

Wang et al. (2014) <sup>[30]</sup> reported that the interplay between aggregation and the formation of mineral-associated SOC is the key to understanding changes in amounts and stability of SOC upon soil erosion and deposition. At the eroding site sub-soils will be exposed after removal of the top-soils. Erosion-induced breakdown of aggregates, for which we used crushing as an analogy, exposed previously protected C with in aggregates at the eroding site, enabling SOC readily to be decomposed [Fig.8c]. The readily soluble components will be released and partially leached along the profile but larger parts of this C rich soil and solutes will be transported and deposited in lower areas of the landscape leading to C enrichment once the material is deposited. Soil erosion and deposition facilitated the modification of aggregate size distribution and associated carbon in the aggregate fractions. After the erosion-induced breakdown of aggregates, the redistribution of aggregate associated SOC led to increased macro-aggregation and macro-aggregate associated C content at the depositional site. However, macro-aggregation does not result directly in smaller C mineralization. Stabilization occurs only by the interplay of aggregation with the formation of mineral-associated SOC. Optimal conditions for macroaggregation as the deposition of organic C rich soil with a high proportion of plant residues are the pre-condition for the formation of mineral-associated SOC.



Fig 8(a): Soil organic C and total N concentrations in aggregates and in micro-aggregates occluded within macro-aggregatesunder conventional farming (CF) and organic farming (OF) [Source: Tian *et al.*, 2018]<sup>[29, 34]</sup>

Fig 8(b): Total organic C and total N in aggregate fractions as influenced by conventional farming (CF) and organic farming (OF) [Source: Tian *et al.*, 2018]<sup>[29, 34]</sup>

Fig 8(c): Conceptual model of the interplay between physical and chemical stabilization of soil organic carbon during erosion and deposition [Source: Wang *et al.*, 2014]<sup>[30]</sup>

Wang *et al.* (2014) <sup>[30]</sup> observed that at both the depositional and the eroding site, the HF represented the most important part of the total SOC at all depths, constituting >80% of SOC [Fig.9a]. The contribution of the HF toSOC was slightly lower at the depositional site than at the eroding site at all depths, indicating the larger contribution of fLF and oLF to SOC at the depositional site. The relative contribution of fLF and oLF to SOC decreased with depth at both sites. No free and occluded light fractions were present at 160e200 cm depth at the eroding site.

Chen *et al.* (2016) <sup>[2]</sup> reported that the SOC concentration decreased with soil depth [Fig. 9b]. In both 0–10 and 10–20 cm, the SOC concentration in the RP treatment was significantly greater than that in the other four treatments, yet no significant differences were found among the other four. In 20–30 cm, there were in general no significant differences among all the rotation systems. For POC, the concentration in the RW treatment was significantly lower than that in the RF, RO, and RP treatments, being 22.6%, 26.0% and 22.7% at 0–10 cm respectively, and 12.0%, 17.0%, and 12.4% at 10–20 cm, respectively. The concentrations of DOC in the RP treatment were significantly greater than in the other

treatments irrespective of the soil depth. By contrast, the DOC concentration in the RF treatment was significantly lower than those in the rotations with plant growth and the decreases in DOC ranged from 46% to 140.0% at 0-10 cm and 36.9% to 80.9% at 10–20 cm soil depths. The concentrations of HWC in the RP and RG rotation systems were significantly higher than those in the RO, RW, and RF rotation systems at the 0-10 cm and 10-20 cm depths. The MBC concentration in RO and RW was significantly decreased compared with RP, RG and RF at the 0-10 cm and 10-20 cm depths. In addition, the values in RG and RP were significantly greater than in the other rotation treatments at the 0-10 cm and 10-20 cm depths. However, no significant differences among the rotations were found at the depth of 20-30 cm [Fig.9b]. The contents of DOC, HWC, MBC, and KMnO4-C in the RP treatment were comparatively higher than in the other treatments. Zhao et al. (2018)<sup>[34]</sup> revealed that the straw return treatments, particularly MR-WR, increased the proportions of mSOM and fine iPOM within small macro-aggregates and micro-aggregates, especially in the 0-20 cm layer [Fig.9c]. The carbon content of iPOM was much lower at 20-40 cm than at 0-20 cm [Fig.9c].



**Fig. 9(a):** Soil organic carbon (SOC) in three density fractions (free light fraction (fLF), occluded light fraction (oLF) and heavy fraction (HF)) from the eroding and depositional site [Source: Wang *et al.*, 2014] <sup>30]</sup>

Fig. 9(b):Effects of different annual straw return rates on soil TOC (A), DOC (B), EOC (C) and MBC (D) contents at the three soil depths [Source: Chen *et al.*, 2016]<sup>[2]</sup>

**Fig. 9(c):** Organic C content (g kg<sup>-1</sup> soil) of the SOC fractions: coarse iPOM, fine iPOM, mSOM, and free LF of small macro-aggregates and micro-aggregates in the 0–20 cm and 20–40 cm soil layers under MR-WR, MR, and WR [Source: Zhao *et al.*, 2018]<sup>[34]</sup>

Poirier *et al.* (2014) <sup>[16]</sup> observed that the residue additions up to 20 g residue-C kg<sup>-1</sup> soil positively influenced LM formation in both soils. The increase in LM formation was two-times greater in the subsoil than in topsoil, but the

maximum amount of LM was greater in topsoil than subsoil [Fig.10a]. The addition of 2.5 and 5 g residue-C kg<sup>-1</sup> soil did not affect SM formation in the topsoil, but promoted SM formation in subsoil. The mass of SM decreased in both soils

with the addition of 10 and 20 g residue-C kg<sup>-1</sup> soil and there was more SM in subsoil than topsoil [Fig.10b]. In both soils, residue input reduced the mass of the m + UP fraction and the effect was more pronounced in the subsoil than topsoil [Fig.10c]. However, there was no significant difference in the mass of WS macro-aggregates between topsoil and subsoil receiving 20 and 40 g residue-C kg<sup>-1</sup> soil. Overall, more new WS macro-aggregates were formed in residue-amended subsoil than topsoil (relative to the un-amended soils), providing a greater macro-aggregation formation rate per unit C added in subsoil [Fig.10b]. In topsoil, WS macro-aggregate formation was highest (28.2 g of >250 mm aggregates per gram of C added) with the lowest residue input (2.5 g residue-C kg<sup>-1</sup> soil). In the subsoil, WS macro-aggregate formation increased to 76.3 g of >250 mm aggregates per gram of C added with residue input of 5 g residue-C  $kg^{-1}$  soil and decreased thereafter [Fig.10b].

When both WS macro-aggregate fractions were combined, the residue-C retained in the POM fraction of soil macro-aggregates showed a significant and positive quadratic relationship with increasing residue inputs [Fig.10c]. In the fine particle-size fraction, there were 2, 1.4 and 1.1 times more residue-C in subsoil than topsoil with additions of 5, 20 and 40 g residue-C kg<sup>-1</sup> soil. The residue-N retained in the POM fraction of soil macro-aggregates showed a positive linear relationship with increasing residue input with a significantly greater slope in subsoil than topsoil due to 1.2 times more residue-N retained at the highest residue input rate [Fig.10c]. The same significant trend was observed for the mass of residue-N retained in the fine particle-size fraction.'



**Fig. 10(a):** Distribution of a) large (LM, >1000 mm) and b) small (SM, 250e1000 mm) water stable macro aggregates, and c) micro aggregates plus un-aggregated particles (m b UP, <250 mm) in topsoil (0e20 cm depth) and subsoil (30e70 cm depth) from a heavy clay soil [Source: Poirier *et al.*, 2014]<sup>[16]</sup>

**Fig. 10(b):** Proportion of water-stable macro-aggregates [large (LM, >1000 mm) b small (SM, 250-1000 mm)] and b) water-stable macroaggregate formation per unit of C added (i.e., D increase in >250 mm aggregates/D C input) in topsoil (0-20 cm depth) and subsoil (30-70 cm depth) from a heavy clay soil [Source: Poirier *et al.*, 2014] <sup>[16]</sup>

Fig. 10(c): Accumulation of residue-C and -N in soil water-stable macro-aggregates (>250 mm) in the form of particulate organic matter (POM, >50 mm) (a, c) and associated with fine particles (<50 mm) (b, d) in topsoil (0-20 cm depth) and subsoil (30-70 cm depth) from a heavy clay soil [Source: Poirier *et al.*, 2014]<sup>[16]</sup>

### Conclusion

The accumulation of C in soil was related to soil aggregation and the distribution of C in aggregates. By significantly improving soil aggregation and associated C content, the potential of CA systems in a

Inter-play between aggregation and the formation of mineralassociated SOC is the key to under-standing changes in amounts and stability of SOC upon depth, occlusion within aggregates, and associated organic carbon fractions. The LF-C and iPOM-C in various aggregate size classes was greatly influenced by conservation tillage in the surface soil layer only. Better soil aggregation was associated with a larger proportion of fine iPOM-C and very labile SOC pool. A reduction in tillage intensity led to a significantly larger SOC accumulation in the surface soil layer (0-5 cm), but not in the subsoil layer. Moreover, both the quantity and surface area of organic inputs critically affected the retention of organic C in the heavy soil fraction. Compared to the coarse-sized residues, fine-sized residues increased labile C (DOC and MBC) and promoted the formation of Fe/Al-bound C and subsequently organic C accumulation in the heavy fraction, particularly aliphatic and carboxylic compounds associated with soil minerals. These results highlight the key role of the contact area between organic materials and soil minerals for the formation of mineral-associated SOC. In topsoil, WS macro-aggregate formation was highest (28.2 g of >250 mm aggregates per gram of C added) with the lowest residue input (2.5 g residue-C kg<sup>-1</sup> soil). In the subsoil, WS macroaggregate formation increased to 76.3 g of >250 mm aggregates per gram of C added with residue input of 5 g residue-C kg<sup>-1</sup> soil and decreased thereafter. The concentration of POC, MBC and HWC were higher under topsoil (0-10 cm) as compared to subsoil (10-20 cm) in CA practices.

Organic carbon concentrations in the <0.053-, 0.053- to 0.25-, 0.25- to 2.0, and >2.0-mm fractions were 14.0, 12.0, 14.4, 24.1% greater, respectively, in CA than in CF. The contents of SOC,LOC, DOC, POC and EOC by 14.73%, 16.5%, 22.5%, 41.5% and 21% in the 0-40 cm soil layer, and by 17%, 14%, 19%, and 30% in the 0-100 cm soil layer. These results suggest that over time, the MBC and MBC-derived C under the fine-sized residue treatment may constitute a significant source of stable SOC through strong physical and chemical bonding to the mineral soil matrix. Conservation management in the North West IGP is important in maintaining soil structure stability and conserving SOC from rapid decomposition with associated organic carbon fractions.

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