

1. Introduction and literature review

1.1. General background

Soil organic matter (SOM) is the sum of all natural and biologically derived organic material found in soil or on the soil surface irrespective of its source, whether it is dead or at any stage of decomposition but excluding the above ground portion of living plants (Baldock and Nelson, 2000). It has been estimated that globally soil contains 1500 Pg of organic carbon which is 4.5 times larger than carbon in biotic pool and 3.3 times than atmospheric carbon (Batjes, 1996; Lal, 2004a). Any small increase in soil organic carbon (SOC) could have huge potential at the global scale to stabilize and store large amounts of carbon in the soil (Smith et al., 2009). Soil organic carbon stabilization and destabilization are interrelated with the stability of soil aggregates (Sollins et al., 1996).

Soil aggregates are the functional unit for soil structure. The primary soil particles form the nuclei of soil structure by the process of flocculation and cementation. Soil aggregates are bound together by transient and persistent binding agents into soil structure (Tisdall and Oades, 1982). Soil organic matter is directly involved in the aggregation process and SOM dynamics in soil aggregates is viewed as the most significant process of SOM stabilization in soil. Soil organic matter dynamics is closely related with the formation of macroaggregates and microaggregates (Angers and Chenu, 1998). By definition aggregates that are $>250 \mu\text{m}$ in diameter are called macroaggregates and aggregates with diameter $<250 \mu\text{m}$ are microaggregates (Edwards and Bremner, 1967). Soil aggregates, especially microaggregates have a unique capacity to store SOC (Elliott, 1986). The pore size and pore distribution of aggregates control access of soil microbes to organic substrate and hence stabilize SOC by physical protection. The primary silt-plus-clay particles also protect soil organic matter by a physico-chemical stabilization mechanism, which is related to adsorption of SOM onto soil minerals. It has been shown that the organic matter protection capacity of soil is closely related with its silt-plus-clay percentage (von Lützow et al., 2006; Hassink et al., 1993). Apart from physical and physico-chemical protection, soil organic matter possesses recalcitrance to the extent that its molecular structure inhibits microbial attack. For instance, cellulose decomposes more rapidly than the lignin and other aromatic ring containing molecules in soil (Baldock et al., 1992). The storage of organic carbon in aggregates is also dependent on land use, soil

characteristics, plant or crop species and climatic conditions.

1.2. Hypotheses and Objectives of the study

The overarching hypotheses of the study were (i) physical protection of SOC is a dominant SOC stabilization mechanism in soil microaggregates over a physico-chemical protection mechanism (ii) land use and soil type determine the relative importance of different stabilization mechanisms. The specific objectives of the research work were to investigate (i) the SOC stabilization in water stable aggregates (ii) microbial decomposition of SOC and its relationship with chemical composition of SOC and pore geometry of microaggregates (iii) the mean residence time of SOC in microaggregates and (iv) the influence of land uses on the distribution and stabilization of SOC in microaggregates.

1.3. Thesis structure

This thesis is written in journal article format and parts of literature review might be repeated in subsequent chapters. The reference style in each chapter is kept journal specific. An introduction and literature review covering the aspects of soil aggregation and SOC turnover is presented in Chapter 1. The SOC stabilization in Alfisols is presented in Chapter 2, 3, 4 and 5. The stabilization of SOC in Oxisols is presented in Chapter 6. The estimation of mean residence time of both Alfisols and Oxisols is presented in Chapter 7.

Chapter 2 examines the distribution of water stable aggregates and physical SOC fractions in macroaggregates, microaggregates and <53 μm fraction under contrasting land uses. The objective of this chapter is to study the SOC stabilization in water stable aggregates under contrasting land uses.

Chapter 3 examines the SOC mineralization rates, sizes of chemical and biological SOC pools and mean residence time of SOC in macroaggregates, microaggregates and <53 μm fraction under contrasting land uses. The objective of this chapter is to study the stabilization of SOC in aggregate size fractions against microbial decomposition and chemical oxidation under contrasting land uses.

Chapter 4 examines the chemical composition of SOC fractions in macroaggregates, microaggregates and <53 μm fraction using solid state ^{13}C NMR under contrasting land uses. This chapter provides relationships between chemical composition of SOC fractions in different aggregate size ranges and SOC stabilization against microbial decomposition and chemical oxidation.

Chapter 5 examines 3D pore geometry of macroaggregates and microaggregates using high resolution X-ray computed microtomography under contrasting land uses. This chapter provides the relationship between 3D pore geometry and SOC stabilization in Alfisols.

Chapter 6 examines the distribution of water stable aggregates and physical SOC fractions in aggregates of Oxisols under contrasting land uses. This chapter also examines respiration rate and 3D pore geometry of different aggregate size ranges. This chapter provides the relationship between 3D pore geometry and SOC stabilization in Oxisols.

Chapter 7 examines the mean residence time of SOC in different aggregate size ranges by measurement of radiocarbon signature.

Chapter 8 provides a summary and synthesis of all the outcomes of the above mentioned chapters, and discusses with future research implications.

The following sections will present a brief review of theories of soil aggregation and SOM dynamics in soil aggregates.

1.4. Mechanism of soil aggregate formation

The theory of microaggregates was put forward by Edwards and Bremner (1967). They proposed that soil consists of microaggregates (<250 μm) bound into macroaggregates (>250 μm) and that microaggregates comprise of clay particles linked by polyvalent cation and humified organic matter bridges.

Tisdall and Oades (1982) extended this view of aggregation at different scales with the aggregate hierarchy concept in which it was postulated that the different binding agents act at different hierarchical stages of aggregation (Fig. 1.1). Primary particles and silt-sized aggregates (<20 μm) are bound together into microaggregates (20-250 μm) by

persistent binding agents such as humified organic matter and polyvalent metal cation complexes. These stable microaggregates are bound together into macroaggregates (>250 μm) by temporary binding agents like fungal hyphae, plant roots and microbial- and plant-derived polysaccharides. Oades (1984) proposed a modification to the concept and postulated that the binding agents that hold the macroaggregate together form a nucleus for microaggregate formation at the center of the macroaggregate. The theory of microaggregate formation within macroaggregate was strongly supported by Angers et al. (1997) and implies that occlusion of SOC in microaggregate takes place after its occlusion in macroaggregate. Therefore, the SOC in microaggregates are older than that of macroaggregates (Angers et al., 1997; von Lützow et al., 2007).

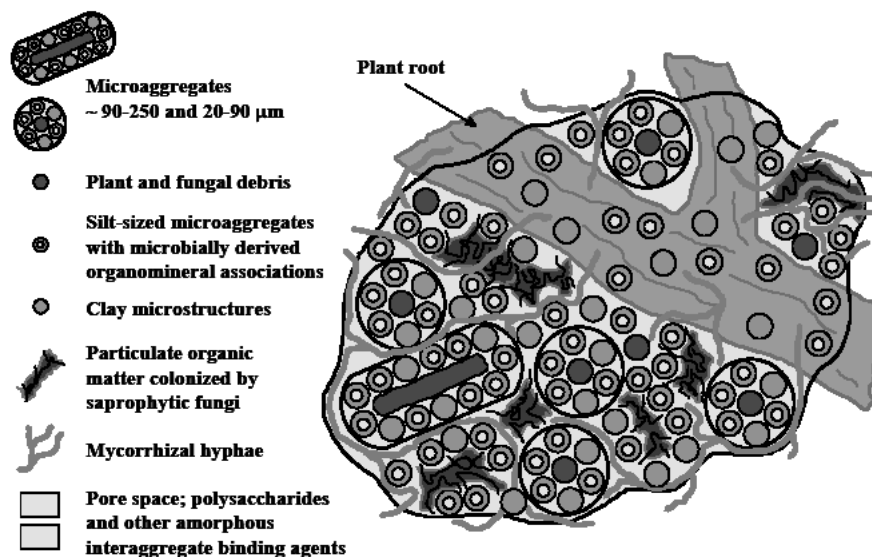


Fig.1.1. Conceptual diagram of aggregate hierarchy (Jastrow and Miller, 1998)

Based on the aggregate hierarchy principle couple of other important concepts emerged to describe aggregate pore size distributions (Elliott and Coleman, 1988; Dexter, 1988). Elliott and Coleman (1988) described four hierarchical pore categories as a mirror image of aggregate hierarchy. The four pore categories are (i) macropores, (ii) inter-macroaggregate pores, (iii) inter-microaggregate pores within macroaggregate and (iv) intra-microaggregate pores.

The porosity exclusion principle that was proposed by Dexter (1988) described the step wise reduction of macro-porosity from the aggregates of higher hierarchical order such as microaggregates. For example, the porosity of microaggregates is lower than

macroaggregates due to the exclusion of pores that are present between microaggregates within macroaggregates.

One of the most significant conceptual models of soil aggregate formation was proposed by Golchin et al. (1994) who showed that particulate organic matter (POM) (fresh plant debris or partially decomposed SOC) acts as a nucleus of microaggregate formation. Particulate organic matter, rich in carbohydrates acts as a centre for microbial activity but adsorbs a coating of mineral particles bound by microbially produced mucilage and metabolites. This protects the organic matter from rapid decomposition. During the initial stage of decomposition of these organic cores, aggregates show high stability. As decomposition progresses within aggregates the concentrations of the more resistant plant parts increases. As this organic core is finally depleted the stability of microaggregates also decreases and upon breakdown the mineral part encrusted with microbial byproducts are released to form stable silt-sized organo-mineral complexes (Fig. 1.2). Lynch and Bragg (1985) also reported that POM is colonized by the microbial population and that microbial byproducts bind the soil primary particles together. The incorporation of crop residues thus has the potential to improve soil structure as the plant fragments become the center of water stable aggregates (Angers et al., 1997). Similar results and emphasis on the long term storage of soil carbon in microaggregate were presented by Beare et al. (1994a, 1994b); Jastrow (1996); Jastrow and Miller (1998); Six et al. (1998); Gale et al. (2000) and Puget et al. (2000).

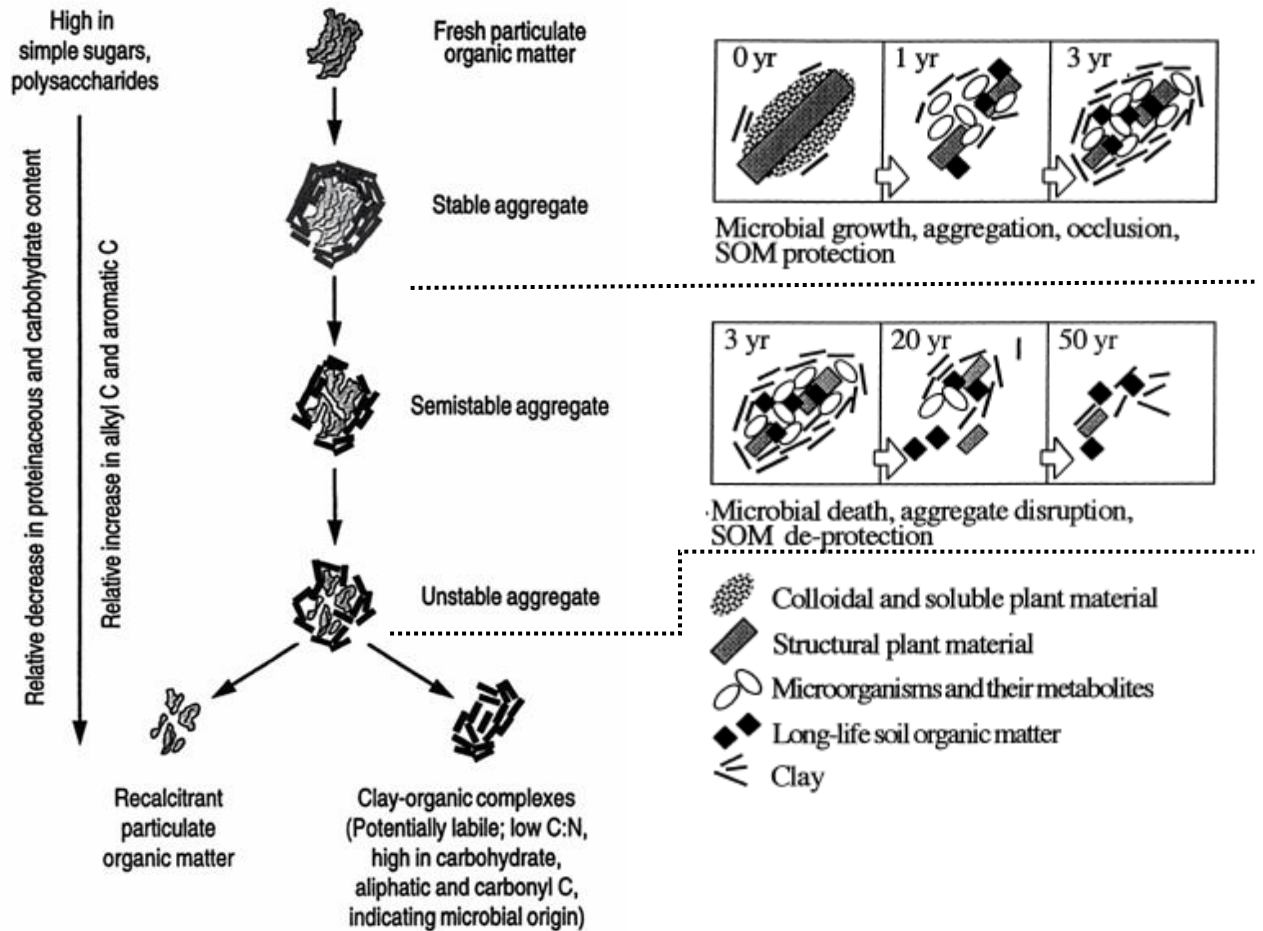


Fig. 1.2. Conceptual model of microaggregate formation by Golchin et al. (1994) (Adopted from Jastrow and Miller, 1998; Balesdent et al., 2000)

Six et al. (2000a) proposed a conceptual model of the life cycle of macroaggregate which describes the creation of new microaggregates within macroaggregates (Fig. 1.3). Microaggregates form and stabilize around fine POM encrusted with microbial products and earthworm mucus and with time it becomes unstable due to cessation of microbial activity. Tillage enhances the turnover of macroaggregates and coarse particulate organic matter which reduces the possibility of formation of microaggregates. The mechanism of aggregate formation described in Six et al. (2002a) is similar to Golchin et al. (1994) but it advances the understanding of relationship between tillage and formation of stable microaggregates.

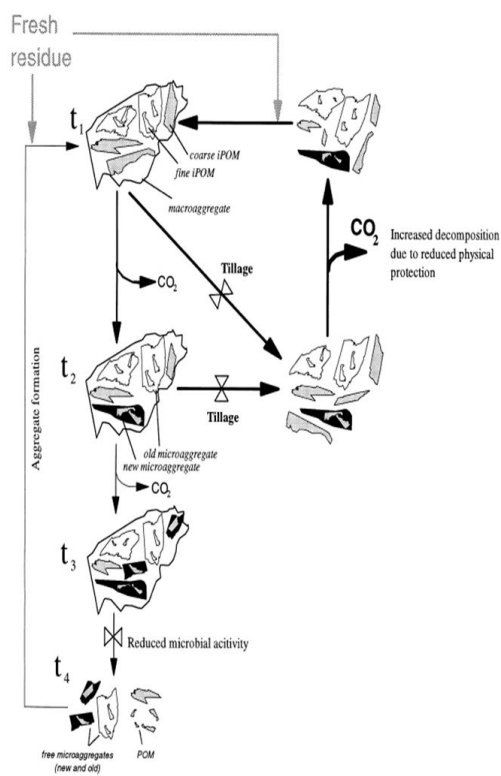


Fig.1.3. The conceptual model of life cycle of a macroaggregate and formation of microaggregates (Six et al., 2000a).

In contrast to the aggregate formation hypotheses proposed by Tisdall and Oades (1982); Oades (1984); Golchin et al. (1994) and Six et al. (2000a), Lehmann et al. (2007) stated that rather than occlusion of organic matter in soil particles, it is adsorption of organic carbon on clay surfaces that initiate microaggregate formation (Fig. 1.4). This model emphasizes the importance of adsorption of decomposition products of plant debris, and microbial metabolites on clay particles for the formation of stable microaggregate. The conceptual model of Lehman et al. (2007) is similar to the mechanism of microaggregate formation proposed by Edwards and Bremner (1967) and strongly relies on the stability of primary organo-mineral complexes (Mikutta et al., 2006; Christensen, 2001). The interaction of SOC with clay is also dependent on types of clay minerals. Deneff and Six (2006) proposed a conceptual model for aggregate formation in illitic and kaolinitic soils. The model illustrated that the aggregate formation can differ in kaolinitic soils due to lower surface charges in kaolinitic compared to illitic soil.

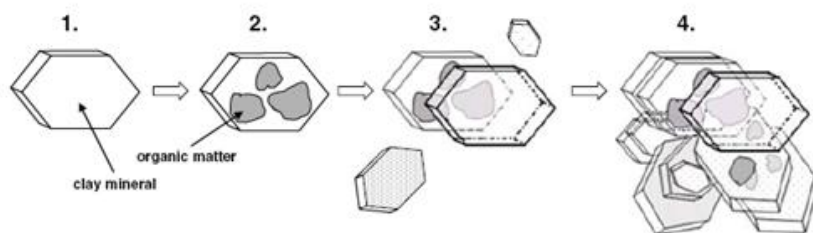


Fig. 1.4. Sandwiched organic matter between clay particles (Lehmann et al., 2007)

Researchers have also emphasized the role that root derived POM plays in microaggregate formation (Puget and Drinkwater, 2001; Rasse et al., 2005). Active root and dead roots can enhance microbial activity and production of aggregate binding agents (Czarnes et al., 2000; Puget and Drinkwater, 2001; Gale et al., 2000). Moreover, the physical enmeshment, compression forces and modification of soil water status caused by penetrating roots can promote aggregation (Denef and Six, 2006; Six et al., 2004).

Besides the major contribution of plant residues, roots and microbes the role of soil fauna (e.g. earthworms and termites) in forming stable aggregates has also been studied. Brown et al. (2000) stated that earthworms mediate soil aggregate formation by burrowing and cast formation. Numerous studies have shown that the stability of earthworm casts is higher than the surrounding soils (Shipitalo and Portz, 1988). Little is known on the role of termites in soil aggregation; however, it is evident that some species have a positive influence on soil aggregate stability. Termite mounds have diverse bacterial and fungal population which enhance SOC decomposition and improve soil microaggregate stability (Holt and Lepage, 2000).

1.5. Land use and aggregate stability

Soil management has a pronounced influence on aggregate size distribution and water stability but the influence depends on the magnitude of soil disturbance (Blanco-Canqui and Lal, 2004). Cultivation of soils under conventional tillage system means plowing, harrowing and mixing of soil and crop residues (Beare et al., 1994b). Compared to conventional tillage, conservation tillage refers to an agronomic system in which at least 30% of soil is covered with crop residues and intensity of soil disturbance is minimal. No-tillage or zero-tillage is a type of conservation tillage (Paul, 2005; Robert and Jack, 2005). Conventional tillage decreased aggregate stability mostly in surface soil (Blanco-Canqui and Lal, 2004; Kodesova et al., 2009). Macroaggregates are less stable

than microaggregates and more prone to disruptive force (Elliott, 1986; Cambardella and Elliott, 1993). Surface soil under conventional tillage, subject to frequent cultivation, undergoes more frequent wetting and drying cycle and experience more disruptive forces from tillage equipments which results breakdown of macroaggregates and the formation of SOC depleted microaggregates (Six et al., 2000c). The deterioration of macroaggregates under conventional tillage liberates SOC from the aggregates and makes it more susceptible for microbial decomposition (Six et al., 2000a, 2000b; Six et al., 2002a, 2002b; Deneff et al., 2001). Six et al. (2000a) showed that microaggregates formed in macroaggregates comprised 47% of macroaggregate weight under no-tillage compared with 27% under conventional tillage. Thus, microaggregate formation and stabilization of carbon appear to be linked with a faster breakdown of macroaggregates which reduces the microaggregate formation within microaggregate and SOC stabilization.

The higher macroaggregate formation under no-tillage or conservation tillage was reported by Beare et al. (1994a, 1994b), Baldock et al. (1987), Guggenberger et al. (1999a), Vaisanen et al. (2005), Bossuyt et al. (2002), Besnard et al. (1996), Kushwaha et al. (2001). John et al. (2005) reported that distribution of aggregate size classes was influenced by land cultivation as tillage destroyed especially aggregates >1000 μm . They found that in the tilled horizon of maize and wheat soils, the most abundant aggregate fractions were the microaggregates (53-250 μm) and macroaggregates (250-1000 μm), respectively. In contrast to tilled soil in un-tilled grassland and forest soils the mega-aggregates (>2000 μm) were most abundant. Martins et al. (2009) concluded that crops have ability to modify soil aggregation status and it is dependent on rooting pattern, root functions and physiology. Monocotyledons with fibrous root system (e.g. barely) and perennial pasture are more effective to promote soil structural stability (Chan and Heenan, 1996; Golchin et al., 1998; Skjemstad et al. (1990); Pulleman and Marinissen, 2004). Paul et al. (2008a, 2008b); Gartzia-Bengoetxea et al. (2009) stated that natural forest contained more macroaggregates than pasture soils.

The key factors leading to differences in aggregate stability between no-tillage and conventional tillage were reported as decline of SOC and progressive destabilization of aggregates under conventional tillage practices. Moreover, SOC input rate also varies between cultivated soils, grassland and forest but the influence of SOC input rate on aggregate stability is not equivocal (Abiven et al., 2009).

1.6. Soil organic matter associated with aggregates under different land uses

Afforestation, conservation farming systems, crop-rotation with pasture species, integrated nutrient management with compost, retention of crop residues etc. can enhance the capacity of soil to sequester SOC (Del Galdo et al., 2003; Lal et al., 2004b). Under such management, SOC can accumulate in soils because of tillage-induced soil disturbances elimination, reduction of erosion losses and addition of large quantities of above ground and below ground biomass to soil. In general, conversion of agricultural land to a more natural land use, such as pasture and woodland reduces the rate of SOC loss from soil (Lal, 2004b).

Macroaggregate stabilization is important for maintaining SOC, because disruption of macroaggregates enhances SOC mineralization. Bossuyt et al. (2002) stated that unprotected SOC was higher under no-till than under conventional tillage because of faster breakdown of macroaggregates which led to faster decomposition of SOC under conventional tillage. Generally, frequency and intensity of tillage have effect on aggregation, organic matter content and biological activity of soils. No-tillage in particular may increase soil organic matter, increase aggregate stability, microbial biomass and earthworm abundance (Doran, 1980; Fonte et al., 2007; Parmelee et al., 1990). Besides, no-tillage enhances bacterial activity and favors fungi over bacterial population (Frey et al., 1999; Guggenberger et al., 1999b). Six et al. (2000a) showed that the inter-microaggregate particulate organic matter concentration was 1.3 g C kg^{-1} in under no-tillage compared with 2.8 g C kg^{-1} under conventional tillage. In contrast, no-tillage had three times more intra-aggregate particulate organic carbon than conventional tillage. Roscoe and Buurman (2003) reported that in Oxisols, conversion of savannas to plow tillage and no-tillage did not change the SOC stock. However, 22% of total SOC was replaced by younger maize derived SOC.

It was also reported that free POM is the most sensitive fraction to change in soil management. The free POM refers to the un-complexed POM that is not incorporated in soil aggregates (Christensen, 2001). The free POM is also referred as light fraction carbon. Tan et al. (2007) observed soils under forest and no-tillage practice retain more light fraction carbon than those under conventional tillage. Concentrations of light fraction carbon in all aggregate classes were significantly higher under no-tillage and forest than under conventional tillage. Of the total SOC pool, silt-plus-clay associated carbon accounted for 76% in conventional tillage and 63% in forest and no-tilled soils.

The lower concentration of light fraction carbon in conventional tillage is also related to lower SOC stock (expressed in kg C m⁻²) under conventional tillage compared to forest and no-tillage practice (Tan et al., 2007).

Soils under pasture are better aggregated and sequester more carbon than intensively tilled croplands because of increased return of residues, reduced soil disturbance and contribution from pasture root system (Percival et al., 2000). Soil aggregation may differ between grasslands and forests. Sharrow and Ismail (2004) estimated that about 90% of organic carbon in pastures is stored belowground where as <40% of organic carbon in forests is stored belowground. The high below ground residues may increase aggregation in pastures. The organic carbon in grasslands is in greater interaction with the soil than in forests because most of the SOC in grassland is stored belowground. The interaction between soil and SOC in grasslands can promote long term SOC sequestration within microaggregates compared to forests. However, the difference in total SOC storage between grassland and forests are often inconsistent (Blanco-Canqui and Lal, 2004). The SOC storage in grasslands can be equal (Corre et al., 1999), higher (Garten and Ashwood, 2002) or lower (Silver et al., 2000) compared to forest. The effect of pastures on SOC sequestration is dependent on pasture species and pasture management (Chan et al., 2010).

Kaiser et al. (2002) investigated two acid forest soils and reported that about 45% of total SOC is transferred to subsurface horizon and become stabilized by organo-mineral association with secondary sesquioxides. Muller and Kögel-Knabner (2009) reported that larger total SOC stocks were found under continuous forest compared with grassland, due to the carbon stored within the organic horizons in the forest. The SOC storage in forest soil is influenced by tree species and management (Del Galdo et al., 2003). John et al. (2005) found that in a spruce stand >50% of SOC was associated with POM but it was only <10% at grassland, maize and wheat sites on silty soils. Similar conclusion on POM in forest soils compared to grassland and cultivated soils was also made by Yamashita et al. (2006); de Alcantara et al. (2004); Besnard et al. (1996). Grunzewig et al. (2007) by measuring ¹³C abundance in afforested and native shrubland soil found that in afforested soil a considerable portion of SOC was derived from older shrubland vegetation. A large part of both old and new SOC was protected by organo-mineral association with soil minerals. The authors also concluded that litter quality had significant influence on litter decomposition rate. They found considerably slower decomposition rate of low quality forest litters compared to litters in shrub land.

1.7. Turnover of soil aggregate associated carbon

Microbes utilize soil organic carbon for energy and for synthesis of essential macromolecules like polysaccharides, nucleic acids etc. In the soil environment microorganisms oxidize organic carbon and produce inorganic CO₂. The rate of carbon turnover or mineralization by microbial communities depends on the nature of organic matter and is mostly controlled by the soil environment. The utilization of soil organic carbon by microbes is directly related to soil respiration. The basal respiration is the steady state rate of respiration in soil which comes from the decomposition or turnover of organic matter. Soil aeration, water content, availability of nutrients, temperature, pH etc., are the most important factors that regulate soil respiration or carbon mineralization rate (Pell et al., 2006).

Generally, carbon mineralization rate decreases with decreasing aggregate size (Jastrow, 1996; Six et al., 2002a, 2002b). The substrate utilization by bacteria was lower in 100-250 µm aggregates compared to >250 µm aggregates which determine the protective capacity of microaggregates (Lupwayi et al., 2001; Mutuo et al., 2006). Skjemstad et al. (1990) estimated that about 32% of more old carbon could be stored in microaggregates (<200 µm) than macroaggregates and the turnover rate could vary from 75-348 years. The calculation of Six et al. (2002b) and von Lützow et al. (2006) provided the view that the mean residence time of organic carbon associated with microaggregates are longer than with macroaggregates. In seven studies reported by Six et al. (2002b) the MRT of carbon associated with macroaggregates was 42±18 years (mean±s.e.) whereas it was about 209±95 years in microaggregates. The turnover rate of carbon associated with the silt-plus-clay fraction was slower (29-38 years) than that associated with sand fraction (13 years) (Feller and Beare, 1997). A similar estimation was also made by Yamashita et al. (2006) who found that the organic matter associated with the <53 µm mineral fraction had a turnover time of about 102 years compared with 69 years in >2000 µm aggregates (Yamashita et al., 2006).

Franzluebbers and Arshad (1997) reported that carbon mineralization rate and microbial biomass were lower in microaggregates than macroaggregates. These authors also reported a difference in microbial biomass and carbon mineralization between zero and conventional tillage. Beare et al. (1994a) reported that macroaggregate protected organic carbon was about 18.8% of the total mineralizable carbon in no-tillage soil but only 10.2% under conventional tillage. The higher amount of particulate organic matter

associated with water stable macroaggregates may be responsible for higher mineralization rate (Elliott, 1986; Puget et al., 2000). Razafimbelo et al. (2008) found the amount of carbon mineralized over 28 days was higher under no-tillage than under conventional tillage and higher in mesoaggregates (20-200 μm) than in macroaggregates (200-2000 μm).

The difference in SOC mineralization rate is also influenced by the method of aggregate separation (Ashman et al., 2003). The lower SOC mineralization rate of microaggregates compared to macroaggregates is generally observed with aggregates which are obtained by wet sieving (Franzluebbers and Arshad, 1997). However, such relationship is not evident in aggregates separated by dry sieving (Gupta and Germida, 1988; Puget et al., 1995) because in dry sieving aggregates disrupts by mechanical agitation and thus hierarchy in the aggregate size ranges cannot be obtained. Ashman et al. (2003) also reported that basal respiration rate of $>2000 \mu\text{m}$ aggregates which were obtained by wet sieving with slaked pretreatment was higher than the $>2000 \mu\text{m}$ aggregates obtained with shaken pretreatment.

Dalal and Mayer (1986) observed that the rate of loss of carbon from the light fraction was 2-17% higher than the heavy fraction which is intimately associated with clay fraction of soil. The higher carbon mineralization of light fraction was also reported by Alvarez and Alvarez (2000). Huygens et al. (2005) observed that the carbon mineralization rate of free macro organic matter separated by density separation was higher than organic carbon associated with mineral fraction of soil. For instance, the carbon mineralization rate constant of light fraction under grassland was 0.15 yr^{-1} whereas the mineralization rate constant of carbon associated with mineral fraction was 0.11 yr^{-1} . Such observations demonstrate the role of physical protection rather than primary recalcitrance of soil organic matter (Huygens et al., 2005). However, other experimental findings have shown that carbon mineralization rate largely depends on plant residue quality and the plant part under investigation. For example, Trinsoutrot et al. (2000) found that shoots and leaves had higher decomposition rate than the root of some common arable crops of temperate region. This may be due to higher suberin content of plant roots (Rasse et al., 2005; Grunzweig et al., 2007). Similarly, Gale and Cambardella (2000) found more rapid mineralization of incorporated residues than roots in soil aggregates.

Plante and McGill (2002) conducted a laboratory incubation experiment to simulate the effect of organic matter addition and tillage intensity on carbon

mineralization rate and found that the mineralization rate decreased with increasing tillage intensity. These authors also observed that aggregate stability of soil decreased but the formation of new aggregates was increased in treatments with high tillage intensity. Therefore, they concluded that the formation of new aggregates might decrease the mineralization rate of added organic matter.

It is generally accepted that the mineralization of organic matter in tropical soils is faster than in temperate soils. Six et al. (2002b) found that the mean residence time of carbon in temperate soil (63 ± 7 years) to be 1.8 times longer than that of tropical soils (35 ± 6 years). In some tropical soils especially in Oxisol and similar soils, where soil aggregation is not important for soil organic matter stabilization, the relationship between aggregate size and carbon mineralization is weak (Mutuo et al., 2006). The weak influence of aggregate classes on soil respiration in Oxisols and similar soils may be attributed to its high clay and sesquioxides contents (Mutuo et al., 2006; Barthès et al., 2008).

1.8. Stabilization of organic matter in soil aggregates

Stabilization means decrease in the potential for soil organic carbon loss by respiration or decomposition (Sollins et al., 1996; von Lützow et al., 2006). On the other hand, recalcitrance comprises molecular level characteristics of organic substances which includes elemental composition, presence of functional groups and molecular conformation. The chemical structure of recalcitrant substance influences their degradation by microbes and enzymes. Sollins et al. (1996) defined stability as the integrated effect of recalcitrance, interactions, and accessibility. Stabilization of soil organic carbon increases with decrease in its accessibility to microbes and extracellular enzymes. The term interaction refers to the intra-molecular linkage between organics and either inorganic or organic substances.

The mechanism of soil organic matter stabilization can be divided into physical, chemical or physico-chemical and biological or biochemical protection (Fig. 1.5) (von Lützow et al., 2006).

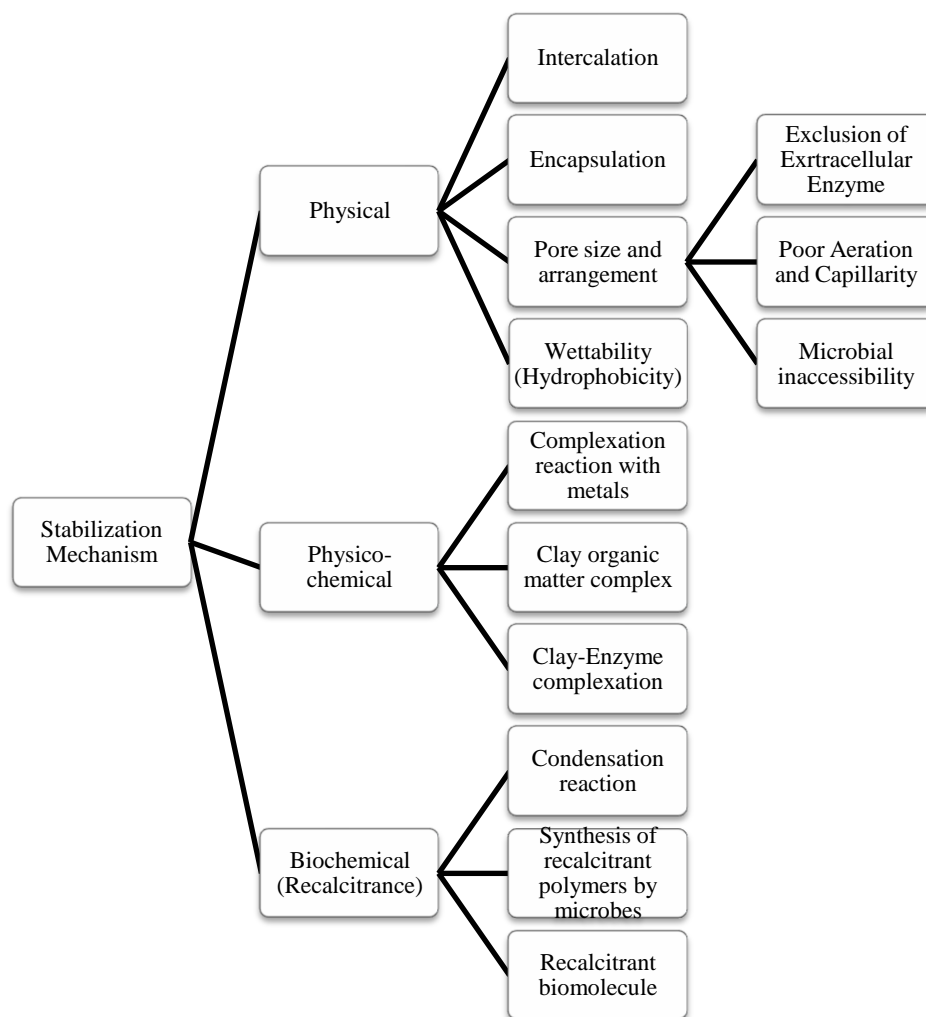


Fig.1.5. Soil organic matter stabilization mechanisms in aggregates (von Lützow et al. 2006; Sollins et al. 1996)

1.8.1. Physical protection

The physical protection of organic carbon by soil microaggregates is well documented (Six et al., 2000c, 2002a, 2002b; Krull et al., 2003; Balesdent et al., 2000). This physical protection results from inaccessibility of soil organic carbon to the microbial community by (i) occlusion of soil organic matter in microaggregate (ii) intercalation of organic matter within phyllosilicates (iii) hydrophobicity of organic matter or reduction of wettability and (iv) encapsulation in organic macromolecules (von Lützow et al., 2006).

The most stable microaggregates are 2-20 μm in diameter (Krull et al., 2003). McCarthy et al. (2008) showed that there is a sharp increase of pore volume at diameters between 0.1 μm and 1 μm (100-1000 nm) in microaggregates. Fungal hyphae are

generally 1-15 μm in diameter (Hudson, 1992) and mycorrhizal hyphae are just few micrometers across. So, fungi are capable of vigorously exploit both macroaggregates and microaggregates (Chenu and Stotzky, 2002; Kong et al., 2010).

Bacteria have dimensions ranging from 0.2-2.0 μm (Bergey and Holt, 1994). Kilbertus (1980) stated that bacteria cannot exist in soil pore smaller than 3 μm . In contrast, van der Linden (1989) showed that pores $<0.2 \mu\text{m}$ in diameter are not accessible to bacteria. Therefore, it is difficult to conclude the limiting pore diameter for accessibility of bacteria. Considering the $<0.2 \mu\text{m}$ pores are not accessible to bacteria, theoretical calculation of van Veen and Kuikman (1990) demonstrated that 95% of the pore space in a silt loam soil would be inaccessible to bacteria. Chenu and Stotzky (2002) estimated that 15% of porosity in sandy soil and 52% in a clayey soil are inaccessible to bacteria. However, Sessitsch et al. (2001) estimated the population of bacteria in various size fractions of soil and concluded that bacterial community structure is affected by particle size and the smaller size fractions host a higher diversity of microorganisms. Higher nutrient availability in smaller size particles may be responsible for such higher bacterial diversities.

van Gestel et al. (1996) stated that the interaction between microbes, organic matter and clay is required for the survival of microbes. Pore size distribution not only controls the accessibility but also faunal predation by pore size exclusion (von Lützow et al., 2006). van der Linden et al. (1989) stated that protozoa and nematodes are excluded from pores $<5 \mu\text{m}$ and $<30 \mu\text{m}$, respectively. Bacterial biomass correlates with the soil volume made up by pores with diameter between 0.2 μm and 1.2 μm where predators like protozoa and nematodes are excluded (Hassink et al., 1993). A similar conclusion was drawn by Sessitsch et al. (2001) who added absence of fungal competition as another reason for higher bacterial diversity in fine pores.

The relationship between microbial activity and water content depends on aeration as well as having adequate water (Rodrigo et al., 1997). A pore size of about 10 μm defines the boundary between free drainage water and capillary water (Kirkham and Powers, 1972). So, in drained moist soils pores $<10 \mu\text{m}$ are often water filled. This implies that anoxia is likely to prevail in the center of microaggregate (Zausig et al., 1993; Rasse et al., 2005; Sextone et al., 1985). This may change the microbial community from aerobic to facultative or obligate anaerobic (Rodrigo et al., 1997). In mineral soils the optimum matric potential for decomposition is between -10 and -50 kPa which approximates the water content at field capacity (Miller and Johnson, 1964). Soils with

very high organic matter content have much lower optimum matric potential ranging from -2 to -30 kPa. The lower limit for microbial activity is around -1500 kPa for bacteria and -5000 kPa for fungi and some actinomycetes (Harris, 1981). At water potential near saturation decomposition become slow due to the absence of oxygen throughout the soil.

The extracellular enzymes that are produced by decomposer microbes must be in contact with the organic substrate to initiate decomposition. In addition to pore size limiting microbial access, it can also limit access by enzymes (Baldock and Skjemstad, 2000). Smaller pores of 0.008 μm are not accessible to exoenzymes (Zimmerman et al., 2004; Mayer, 1994) and organic matter in these pores is therefore protected against decomposition. McCarthy et al. (2008) proposed that pore-filling by organic matter (occlusion in pores), largely in submicron pores is an important mechanism of protection of organic matter in microaggregates. Schimel and Weintraub (2003) stated that “the key to SOM preservation derives from the economic rules of return on investment microbes get for producing exoenzymes. Any process that limits the access of the enzymes to organic matter reduces net energy gain and promotes microbial starvation”. “Although microbial enzymes and hydrolysis products might follow pathways through pores that are not filled with organic matter to reach more distant pools of organic matter, a network of organic matter filled pores could increase the tortuosity and reduce the rate of delivery of assimilable nutrients to the microbes” (McCarthy et al., 2008). Such kinetic hindrance makes microbial enzyme production energetically unfavorable, which in turn affects microbial growth and survival (Simoni et al., 2001; Allison, 2005; Allison and Vitousek, 2005; Ekschmitt et al., 2005). Therefore, “knowledge of the biophysical modifications of the constantly changing pore storage capacities, pore connectivity, and pore blockage of heterogeneously distributed pore pathways should contribute to understanding of the microbial processes at the interfaces within soil aggregates” (Smucker et al., 2007).

1.8.2. Physico-chemical protection

Physico-chemical or chemical protection of soil organic matter arises from the interaction of different types of organic carbon with clay particles. The types of organic matter that are adsorbed on clay particles can range from simple organic acids to complex bio-macromolecules such as extracellular enzymes, suberin, DNA etc. (Sollins et al., 1996; von Lützow et al., 2006; Rasse et al., 2005; Violante et al., 2000; Cai et al., 2006).

Sorption of organic carbon on clay particles is one of the mechanisms of organic matter stabilization in soil (Chenu and Plante, 2006; Curry et al., 2007; Kennedy

et al., 2002). Sollins et al. (1996) reviewed sorption, precipitation and complexation reaction of organic matter with clay surfaces. The capacity of soil to protect organic matter increases with increasing clay content (Hassink, 1997; Razafimbelo et al., 2008; Zinn et al., 2007; Christensen, 2001). Based on Hassink (1997) calculation, Six et al. (2002a) showed a linear relationship between silt-plus-clay content of soil to its silt-plus-clay associated organic carbon.

Jones and Edwards (1998) showed that mineralization of sorbed organic carbon on kaolinite, illite and mixed clay was lower than free organic matter. These authors observed greatest reduction in mineralization when organic carbon is sorbed on iron hydroxide. It has been reported in several investigations that the mean residence time of organic carbon is high in silt-plus-clay fraction compared to macroaggregates and microaggregates (Liao et al., 2006; Sollins et al., 2006). Chenu and Plante (2006) considered clay bound organic matter to be more resistant to microbial decomposition and the carbon stabilization of soil is also dependent on fine particle class, texture, mineralogy and cation such as calcium availability (Six et al., 2002b).

Soil mineralogy is a primary control of the relationship between the surface area of adsorption and the amount of organic matter adsorbed (Kaiser and Guggenberger, 2003). Organic matter associated with kaolinite decomposes more slowly than the carbon associated with smectite or illite (Wattel-Koekkoek et al., 2003; Deneff and Six, 2005; Deneff et al., 2004). Sorption to the surfaces of hydroxides and precipitation by Al and Fe can stabilize organic carbon (Kögel-Knabner et al., 2008a, 2008b; Eusterhues et al., 2005; Zinn et al., 2007) because complexation of monomeric and polymeric organic compound with Fe and Al oxides and hydroxides reduces its degradation rate *in vitro* (Martin et al., 1966).

The surface area of clay can be used to predict the extent of organic matter-clay interaction. But surface area of the minerals is not a good predictor of organic matter and mineral interaction in soil because of its discontinuous coverage (Kögel-Knabner et al., 2008b). Mayer (1994) showed that organic matter distributed on the clay surface in a single layer. Ransom et al. (1997) presented evidence of patchy surface adsorption of organic matter on clay which was supported by several other investigators (Kaiser and Guggenberger, 2003; Curry et al., 2007). However, in a recent work Wagai et al. (2009a) showed that most of the specific surface area of clay is completely covered by organic matter.

1.8.3. Biochemical recalcitrance

The decomposition rate of biomacromolecules depends on the nature of bonding within the macromolecules. The presence of bonds which can be broken by hydrolysis (e.g. ester, glycoside, ether, peptide and C-N bonds) makes the organic molecule more susceptible to decomposition. Hydrolytic enzymes such as cellulase, protease, chitinase etc. are abundant in soil. The natural polymers such as lignin is resistant to decomposition because it contains aromatic structures and has arylglycerol- β -arylether (β -O-4) linkages (Derenne and Largeau, 2001; Kögel-Knabner, 2002). The aromatic structure of cutin and suberin also gives these molecules resistance to decomposition (Derenne and Largeau, 2001).

Moreover, hydrophobicity in organic molecules like lipids may be responsible for its slower decomposition rate (von Lützow, 2006). With the advent of modern nuclear magnetic resonance (NMR) techniques it has become possible to relate the decomposition rate with to functional groups present in decomposing organic substrates. According to Baldock et al. (1997) the ratio between alkyl to O-alkyl carbon tends to increase during decomposition. Lignin was not necessarily selectively preserved and it was revealed that during decomposition the aromatic carbon content may increase, decrease or remain unchanged. Thus, relative rate of decomposition can be predicted by using the presence or absence of functional groups in organic matter.

Berg and McClaugherty (2008) focused on the diversity of microorganisms especially bacteria and fungi and their capacity to degrade natural polymers such as cellulose, hemicelluloses and lignin. Marschner et al. (2008) concluded that mean residence time of lignin in arable soils may not be greater than 20 years. The authors further stated as 90% of lignin input degraded then refractory nature of this molecule should be reconsidered. Similarly, the recalcitrance of lipids (Bol et al., 1996), catechol, phospholipids cannot be explained by selective preservation unless the physico-chemical preservation is considered (Marschner et al., 2008).

Another very crucial point is that the presence of a particular organic material in soil may not always be due to its preservation rather it may be due to the recycling of the product. The polysaccharides and biological molecules produced by variety of soil microbial processes are similar in nature and identification of precursors may be critical for determining the recalcitrant nature of any organics in soil (Gleixner et al., 2001).

1.9. Evaluation of soil organic matter stabilization in aggregates

Generally, SOC stabilization mechanisms can be grouped into (i) inaccessibility of SOC to microbes (ii) adsorption of SOC on clay surfaces and (iii) biochemical recalcitrance. These stabilization mechanisms produce different SOC pools with different chemical and biochemical characteristics and turnover time (von Lützow et al., 2008; Kögel-Knabner et al., 2008a). Based on the turnover time SOC pools are broadly subdivided into (i) active (ii) slow or passive and (iii) stable or inert (von Lützow et al., 2008). However, the study of stabilization mechanisms is quite difficult because all these mechanisms at any environmental settings operate in soil simultaneously, and are greatly influenced by soil characteristics, SOM composition and microbial community structure and function. Various fractionation protocols have been developed to isolate SOC with different turnover time from soil aggregates. It is generally accepted that SOC that is occluded in macroaggregates and microaggregates or adsorbed on silt-plus-clay particles is more stabilized and comprises the passive and stable SOC pool (Six et al., 2002a; Sohi et al., 2001). In addition to fractionation, chemical characterization of SOM is useful to understand the selective preservation of organic compounds during SOM decomposition in soil aggregates (Kögel-Knabner et al., 2008b; Rumpel et al., 2004). The examination of pore geometry of soil aggregates provides vital clues for physical protection of SOC inside soil aggregates due to spatial inaccessibility of SOC to microbes (Young et al., 2008). The following sections provide a brief review of techniques used to study SOC stabilization.

1.9.1. Soil organic matter fractionation

Historically soil organic matter was studied in detail from the view point of formation and characterization of humic substances. But during the last 2-3 decades soil organic carbon research was more focused on the development of conceptual model of SOM decomposition and stabilization in relation to aggregate turnover in soil. Techniques have been developed to describe SOC fractions or pools in soil.

1.9.1.1. Physical fractionation

The basis for physical fractionation of SOC is that the physical architecture of soil aggregates controls the stabilization and destabilization of SOC. This technique utilizes the conceptual models of aggregate formation and SOC decomposition. Generally,

physical SOC fractionation involves flotation of un-complexed SOC which is often termed the light fraction or free light fraction or free POM. The density of the light fraction usually varies between 1.5-2.0 g cm⁻³. After flotation and separation of the light fraction, the heavy mineral matrix is dispersed either by sonication or by chemical methods to release the particulate organic carbon (POC) from aggregates. Particulate organic carbon can be further subdivided into POC between microaggregates inside macroaggregates and POC inside microaggregates. Operationally POCs are determined by sieving of dispersed aggregates. A major portion of SOC in aggregates is associated with silt-plus-clay fractions and often termed mineral associated SOC or the organo-mineral complex or primary particle associated SOC. Although techniques used for physical fractionation vary, standard physical fractionation protocols can be found in Elliott and Cambardella (1991), Christensen (1992 & 2001), Golchin et al. (1994), Six et al. (1998), Puget et al. (2000), Sollins (2006), Gregorich et al. (2006), Wagai et al. (2009b), Christensen (1992) and Kögel-Knabner et al. (2008b). However, these fractionation schemes are unable to isolate unique SOC pools with unique turnover times (von Lützow et al., 2007; Rethemeyer et al., 2004; Smith et al. 2002). Crow et al. (2007) reviewed the limitations of density fractionation techniques and concluded that care should be taken to loss of organic carbon and soil dispersion during fractionation of SOC with high density liquid such as sodium polytungstate.

1.9.1.2. Biological fractionation

The biological fractionation of SOC is based on the principle that the microbes mineralize easily decomposable SOM first and leave the relatively recalcitrant portion (Denef et al., 2009). Some argue that as SOM decomposition is a microbial process, biological fractionation should better describe its dynamics. The active or labile pool represents soil microbes and microbial products; slow pool includes resistant plant materials and physically and chemically protected SOC (Parton, 1996). However, long times are required for such fractionation and it is difficult to mimic the natural conditions. The most commonly used method to relate soil carbon dynamics with microbial function is by measurements of basal respiration rate. Basal respiration rate is usually measured by laboratory incubation without substrate addition (Kutsch et al., 2009). Long term laboratory incubation is also used to estimate SOC mineralization rate, microbial labile and passive SOC pools (Collins et al., 2000; McLauchlan and Hobbie, 2004). Exponential models are frequently fitted to carbon mineralization in soil (Stanford and Smith, 1972;

Riffaldi et al., 1996). The single pool models can be a hyperbolic or exponential which assume that soil composed of homogenous SOM pool (Levi-Minzi et al., 1990; Alvarez and Alvarez, 2000; Pineiro et al., 2006). Two or three pool models assume SOC pools with different decomposition rates (Paul et al., 2001; Riffaldi et al., 1996). The estimation of SOC mineralization rate and turnover time of different SOC pools are generally carried out by two or three component kinetic models based on laboratory incubation data. Paul et al. (2001) considered that analytically derived SOC pool sizes are useful to compare management and ecosystem processes for a particular soil type. Moreover, laboratory incubations allow characterization of SOC mineralization and calculation of turnover time in a controlled environment and controlled interaction of SOC with nutrients, temperature and moisture (Hart et al., 1994; Paul et al., 2006; Torn et al., 2005; Dutta et al., 2006; Reichstein et al., 2005). Other options for biological fractionation are isotope labeling (^{13}C , ^{14}C), ^{13}C natural abundance measurement and bomb- ^{14}C measurement (Balesdent, 1987; Flessa et al., 2008; Trumbore, 2009; Rethemeyer et al., 2004; Luo and Zhou, 2003).

Atmospheric testing of nuclear weapons mostly in the 1950s and 1960s produced a spike of $^{14}\text{CO}_2$ in the atmosphere, which entered terrestrial ecosystems through plants and subsequently enriched SOC radiocarbon. For SOC pools which constantly receive new carbon inputs from plants and lose carbon through decomposition, the $^{14}\text{C}/^{12}\text{C}$ ratio in a given organic matter pool reflects both the rate of decomposition and radioactive decay (Torn et al., 2009). Several models have been formulated to estimate turnover rates of SOC fractions (Balesdent, 1987; Harkness et al., 1991; Cherkinsky and Brovkin, 1993; Hsieh, 1993; Trumbore et al., 1996). The estimation of SOC turnover rate from decadal to millennial time scale is one of the advantages of radiocarbon measurement (Wang and Hsieh, 2002). However, the uncertainty related to the model assumptions is a potential problem in using such approaches (Bruun et al., 2005). Moreover, Trumbore (2009) stated that the decline of present day atmospheric radiocarbon would make it difficult to use of bomb-radiocarbon data to study SOC dynamics.

1.9.1.3. Chemical fractionation

The chemical fractionation is used to extract or isolate SOC fractions of similar properties and dynamics. The most common extraction technique used is determination of water extractable dissolve organic matter (DOC). Both cold distilled water and hot water is used to isolate DOC from soil (Chantigny, 2003; Leinweber et al., 1995). Other chemical methods such as potassium permanganate (KMnO_4) are also used to extract

labile SOC (Blair et al., 1995; Tirol-Padre and Ladha, 2004). The acid hydrolysis of SOC by 6N HCl is used to isolate recalcitrant or stable SOC (Stewart et al., 2009). The portion of SOC that is resistant to chemical oxidation is referred to as inert SOC. A range of chemical oxidizing agents such as hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl), disodium peroxodisulphate ($Na_2S_2O_8$) are used to isolate stable SOC (Plante et al., 2004; Zimmerman et al., 2007; Helfrich et al., 2007). However, Jagadamma et al. (2010) concluded that the efficiency and also nature of SOC isolated by various oxidants varies considerably.

1.9.2. Chemical characterization of SOC

Soil organic matter is a heterogeneous mixture of organic macromolecules existing within a complex soil system and is very difficult to characterize (Swift, 1996). It has historically been characterized into operationally defined humic substances based on chemical extractions (Denef et al., 2009). But the focus of SOC dynamics in soil aggregates in relation to SOC stabilization, demands more specific chemical characterization of physically isolated SOC pools (Kögel-Knabner et al., 2006). Recent developments in analytical techniques such as nuclear magnetic resonance (NMR) and pyrolysis mass spectrometry (Py-MS) have allowed characterizing SOC without chemical extraction. In NMR spectroscopy the nuclei of certain atoms in a sample are excited by interactions with radio frequency electromagnetic radiation. The excitation is followed by relaxation and the absorbed energy is lost again from the nuclei at specific radio frequencies dependent on the chemical bonding environment of the nuclei, which are then converted into a normal spectral presentation usually by Fourier Transformation (Tan, 2005). Among the various techniques in NMR spectroscopy the most useful tool to investigate SOC fractions is ^{13}C solid state NMR (Wilson, 1987). Both cross-polarization magic angle spinning NMR (^{13}C CPMAS) and direct polarization (Bloch Decay) NMR are used for characterize SOC (Baldock et al., 1990, 1992; Golchin et al., 1994, 1995; Skjemstad et al., 1996, 1999; Arshad et al., 2011; Dieckow et al., 2009; Gregorich et al., 1996; Helfrich et al., 2006; Kölbl et al., 2004; Steffens et al., 2011). A Number of methodological investigation have been carried out to improve the identification and quantification of organic carbon by NMR (Smernik and Oades, 2000, 2001, 2002, 2003; Smernik, 2006; Skjemstad et al., 1994; Metz et al., 1996; Mao and Schmidt-Rohr, 2004).

Most biomacromolecules present in soil are derived from plants and microbes (Kögel-Knabner, 2002). The dominant classes of plant derived macromolecules are

polysaccharides, lignins, tannins, lipids, cutin and suberin. A few other macromolecules such as chitin, β -glucan, chitosan, D-galactosamine polymer, polyuronides, melanin and murein are originated from fungal and bacterial cells (Kögel-Knabner, 2002). Aspects of their structural composition of SOC regardless of their origin can be determined using NMR spectroscopy by identifying specific NMR spectral regions which represent different functional groups of SOM (Table 1.1).

Table 1.1. The major functional groups and their associated carbon compound (simplified from Baldock et al., 1992; Kögel-Knabner, 2002)

Group	Carbon form	Major compound
Alkyl	Methyl, methylene, methane and quaternary carbon	Lipids, Cutin, Peptides etc.
O-Alkyl	Oxygenated alkyl, alkyl-amino, methoxyl, acetal and ketal carbon	Carbohydrates, Proteins, Hemicellulose etc.
Aromatic	Protonated and carbon substituted aromatics and unsaturated and oxygenated aromatics and unsaturated carbon	Lignin, Tannin etc.
Carboxyl	Carboxyl carbon, esters and amides	Mainly from protein, Lipid, Cutin etc.

Generally, the percentage of O-alkyl carbon is highest in SOM followed by alkyl carbon, aromatic carbon, carbonyl and amide carbon (Denef et al., 2009). However, the analysis of different SOC fractions shows an increasing trend of alkyl carbon from light fraction to occluded particulate organic matter (Kölbl et al., 2005; Steffens et al., 2011; Golchin et al., 1998). Furthermore, the low SOC concentration in isolated fractions is one of the major problems in studying of SOC stabilization by solid state ^{13}C NMR. Other constraints are related to the identification of specific organic compounds, signal overlap of a specific functional group derived from two different macromolecules, presence of paramagnetic nuclei in soil etc. (Mao and Schmidt-Rohr, 2004; Smernik and Oades, 2002; Denef et al., 2009).

1.9.3. Measurement of pore geometry by using X-ray microtomography

The X-ray computed microtomography (μ CT) is a non-destructive imaging technique at a micron level spatial resolution. Simply, μ CT is based on adsorption of X-ray through the object it passed and reconstruction of three dimensional images from two dimensional projection images (Landis and Keane, 2010). Soil structure consists of three dimensional networks of macropores and such networks are also distributed into soil aggregates either continuous or discontinuous to the field scale macropore system (Perret et al., 1999; Lindquist, 2002). The inherent heterogeneity of soil structure and pore networks plays an important role in microbially mediated processes in soil such as SOC stabilization (Young et al., 2008). The decomposition of SOC in soil aggregates depends on pore connectivity, oxygen and water availability. The physical protection of SOC in aggregates is a complex phenomenon because accessibility, microbial activity, oxygen and water availability all are interconnected and depends on aggregate scale pore network (von Lützow, 2006; Young et al., 2008; O'Donnell et al., 2007).

Research has been carried out to characterize pore networks in soil using μ CT images. These investigations provide great insights on pore diameter, spatial variability, pore connectivity, fractal dimension, etc. Moreover, the influence of tillage, organic matter incorporation on pore geometry of soil can also measured by μ C (Vogel and Roth, 2001; Nunan et al., 2006; Peth et al., 2008; Ngom et al., 2011; De Gryze et al., 2006; Kravchenko et al., 2011; Blair et al., 2007; Luo et al., 2010). Other investigations have focused on SOC distribution, microbial interaction at pore scale and integration of microbial and soil structure models (Smucker et al., 2010; Ngon et al., 2011; Kravchenko et al. 2010; Monga et al., 2008; Wu et al., 2004). Microtomography images have also been used to develop pore-scale models to investigate water nutrient and contaminant flow through soil which might help to understand SOC stabilization (Altman et al., 2005; Dal Ferro et al., 2012; Ovaysi et al., 2011). However, the size of soil sample possible to scan with μ CT is ≤ 2 mm which is considered a significant limitation of this technique. Moreover, the identification of SOC and microbes in soil or in aggregates is still not possible with this technique (O'Donnell et al., 2007).

1.10. Conclusion

Despite the large body of scientific literature on soil aggregation and SOC dynamics, the mechanisms of SOC stabilization are still only partially understood (Sollins

et al., 2007). The problems of understanding SOC stabilization and its turnover are largely due to methodological limitations inherent in physical, chemical and biological SOC fractionation techniques that have so far been developed (Rethemeyer et al., 2004; Smith et al. 2002; Crow et al., 2007). Other problems of understanding SOC stabilization are (i) the soil spatial and aggregate scale variability (ii) influence of combination of soil properties and climatic variations and (iii) soil management. Therefore, the interaction of these factors at field scale creates soil and site specific importance of specific stabilization mechanism (Spielvogel et al., 2008; Goebel et al., 2009). Several authors have suggested applying different SOC fractionation techniques and characterization methods in combination to identify SOC stabilization mechanisms (Trumbore, 2009; Crow et al., 2007; Denef et al., 2009). Moreover, advances in X-ray computed microtomography and application of other techniques to investigate the distribution of organic carbon and microbes in soil have the potential to elucidate more clearly SOC stabilization mechanisms. Still much research is needed firstly to better match physically separated SOC fractions and SOC pools determined by biological and chemical characterization and secondly to link SOC mineralization with chemical structure of SOC and pore geometry of aggregates.

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Chapter 2

Storage of soil organic carbon in aggregates and size-density fractions under contrasting land uses

*Manuscript to be submitted to
Australian Journal of Soil Research*

Chapter 3

Biological and chemical carbon pools of soil aggregates under contrasting land uses

Manuscript to be submitted to

Soil Biology & Biochemistry

Chapter 4

Characterization of soil organic matter in aggregates and size-density fractions by solid state ^{13}C CPMAS NMR spectroscopy

Manuscript to be submit be

Soil Biology & Biochemistry

Chapter 5

3D pore geometry of soil aggregates under contrasting land uses using X-ray computed tomography

Manuscript to be submitted to

Plant and Soil

Chapter 6

Soil organic carbon fractions in aggregates under contrasting land uses in relation to pore geometry of aggregates in two Oxisols

Manuscript to be submitted to

Geoderma

Chapter 7

Mean residence time of soil organic carbon in aggregates under contrasting land uses based on radiocarbon measurements

Manuscript to be submitted to

Radiocarbon

8. General discussion and conclusion

In this PhD thesis the role that microaggregates play in the stabilization of soil organic carbon (SOC) has been investigated. The physical, chemical and biological SOC fractions in macroaggregates, microaggregates and the <53 μm fractions were characterized. The chemical composition of SOC associated with various size fractions of aggregates was investigated by ^{13}C CPMAS solid state NMR. The turnover time of SOC was also estimated by measuring the ^{14}C bomb radiocarbon signature in macroaggregates and microaggregates. A quantitative evaluation of pore geometry of soil aggregates was carried out by X-ray computed microtomography (μCT). These measurements were conducted on two types of soils namely Alfisols and Oxisols.

8.1. Soil organic matter stabilization in Alfisols

8.1.1. Key findings

Aggregate associated SOC decreased with decreasing aggregate size and suggested the presence of an aggregate hierarchy. Among the SOC fractions LF-C and cPOC together contained only 10% of aggregate associated SOC. It was estimated that on average 19% of aggregate-SOC was present as fPOC in both macroaggregates and microaggregates. The LF-C and fPOC were sensitive to land use change. The C/N ratio of fPOC in macroaggregates and microaggregates were similar which indicated that fPOC in both aggregates were at similar stage of decomposition. The mSOC contained on average 50% of aggregate-SOC and had lowest C/N ratio of all SOC fractions. Land use did not have significant influence on mSOC which implies that significant amount of SOC could be stored in aggregates even in crop-pasture rotation (Chapter 2).

The basal respiration rate (BSR) of <53 μm fractions was 39% lower than macroaggregates and microaggregates. The sizes of active and slow pool (expressed in percent of aggregate associated SOC) were also higher in the <53 μm fractions. However, stable SOC (percent of aggregate associated SOC) was higher in macroaggregates and microaggregates than <53 μm fractions. The mean residence time (MRT) of slow SOC pool was longer in the <53 μm fraction compared to that of macroaggregates and microaggregates in all sites. Among the land uses native pasture had higher basal respiration rate, active and slow pool as compared with crop-pasture rotation.

But the MRT of slow pool was longer in crop-pasture rotation and woodland compared to native pasture (chapter 3).

The major constituent of SOC in macroaggregates, microaggregates and <53 μm fractions was O-alkyl carbon which represented 44-57%, whereas alkyl carbon contributed 16-27%. There was a concomitant increase in alkyl carbon content in aggregates from macroaggregates to the <53 μm aggregates with decrease in O-alkyl carbon content. The results suggest that SOC associated with <53 μm aggregates was more decomposed than that of microaggregates and macroaggregates. The degree of decomposition varied in order of mineral associated SOC > fine particulate SOC > coarse particulate SOC > light fraction. Although the effect of land use on SOC composition was not strong, the composition of litter in woodland seemed to influence the SOC decomposition in aggregates (Chapter 4).

Microaggregate porosity was lower by 54.4% and the pore connectivity was lower by 22.1%, when compared to that of the macroaggregates. However, small isolated pore volumes were more common (~80%) in microaggregates. Moreover, fractal dimension of macroaggregates and microaggregates showed high pore heterogeneity existed in microaggregates. On average, high macroaggregate and microaggregate porosity and pore connectivity were found in crop-pasture rotation and native pasture, respectively (Chapter 5).

Percent modern carbon in both macro and microaggregates were >100 which indicated presence of post-bomb carbon in soil. The MRT of SOC in microaggregates was similar to that of macroaggregates. The estimated MRT of stable pool in microaggregates was 897 years longer than macroaggregates. However, the longer MRT of aggregate associated SOC and stable pool was not observed under woodland and crop-pasture rotation, respectively. Among the land uses woodland had higher MRT of SOC in soil aggregates compared to native pasture and crop-pasture rotation (Chapter 7).

8.1.2. Synthesis

This study showed that microaggregates could occur inside macroaggregates (bound-microaggregates) and could also be isolated as free-microaggregates by wet sieving. The adsorption of SOC on the silt-and-clay fraction (mSOC) stabilized it SOC against microbial decomposition and chemical oxidation. SOC in microaggregates was stabilized mainly by physico-chemical protection mechanism. However, fPOC also became physically protected due to low pore connectivity and accumulation of alkyl carbon in microaggregates. Low pore connectivity rather than inaccessibility was principal factor of physical protection of SOC in microaggregates. These findings are elaborated in the following sections.

8.1.3. Microaggregates occur within macroaggregates

In this PhD thesis, the >2000 μm aggregates were fractionated into macroaggregates, microaggregates and the <53 μm fraction by wet sieving with slaking and shaking pre-treatments. The isolated microaggregates and <53 μm fractions were dislodged from macroaggregates by the influence of slaking, shaking and sieving. The SOC fractions associated with macroaggregates and microaggregates were separated using the fractionation scheme of Six et al. (1998).

The macroaggregates and microaggregates were scanned using X-ray microtomography (Chapter 5). The X-ray microtomography image of a macroaggregate showed the presence of condensed soil matrix (indicated by solid arrows) in between pore systems (grey patches) of a macroaggregate (Fig. 8.1). Young et al. (2001) stated that well defined soil aggregates should have clear distinction between macro-pores and fine structured soil matrix. So, the condensed soil matrix around pore systems of macroaggregates could be considered to be microaggregates and it can also be concluded that the separated macroaggregates were consisted of microaggregates. The microaggregates contained within macroaggregate were called bound-microaggregates and the isolated microaggregates isolated by wet sieving were called free-microaggregates. Although fPOC, mSOC and alkyl/O-alkyl carbon ratio were higher, the MRT (both incubation and radiocarbon measurement) of SOC was similar in free-microaggregates compared to macroaggregates. The similar MRT of both free-microaggregates and macroaggregates could be due to the presence of bound-microaggregates inside macroaggregates. In chapter 5 it has been demonstrated that porosity and pore connectivity of microaggregates was lower than macroaggregates. It can be also assumed that bound-microaggregates in macroaggregates might also have low porosity and pore connectivity similar to free-microaggregates and exhibit similar SOC protection. So, the results of the current study reinforced the prevailing hypothesis that microaggregates whether free or bound stabilize SOC (Six et al., 2002a, 2002b).

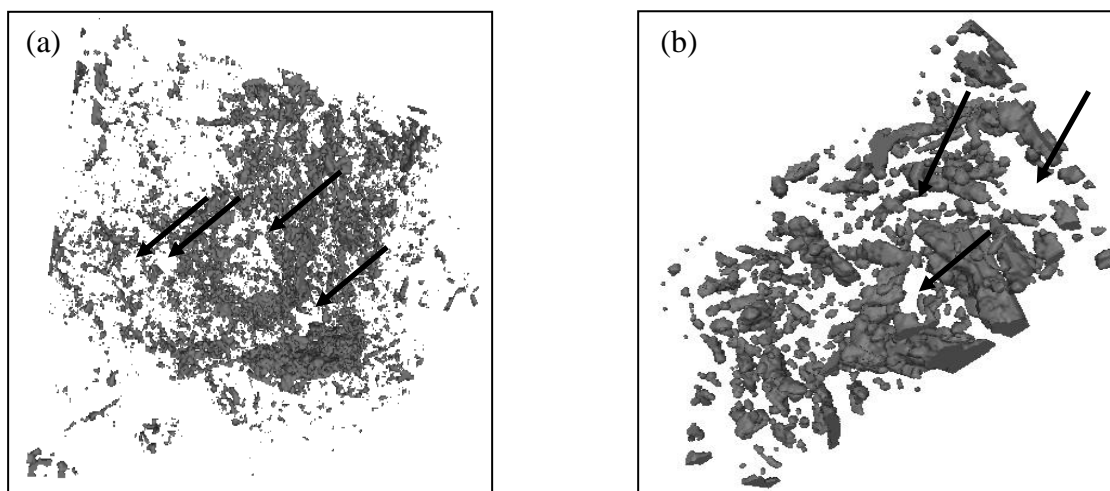


Fig. 8.1. X-ray microtomography images of 3D pore network of two representative macroaggregates of Alfisols. The pores are indicated in grey and soil matrix in white area between grey. The black arrows showing the locations of microaggregates in macroaggregate

8.1.4. SOC stabilization

8.1.4.1. *Physico-chemical stabilization protects SOC from microbial decomposition and chemical oxidation*

Sizes of biological and chemical SOC fractions of aggregates were measured in Chapter 3. The MRT of SOC associated with macroaggregates, microaggregates and $<53 \mu\text{m}$ fractions were also estimated. The longer MRT_s of $<53 \mu\text{m}$ fractions compared to that of macroaggregates and microaggregates suggested that the SOC that adsorbed on silt-plus-clay particles was more protected against microbial decomposition. The stable SOC fraction (resistant to hydrogen peroxide oxidation) was higher in macroaggregates and microaggregates compared to that of $<53 \mu\text{m}$ fraction. The stable SOC of macroaggregates and microaggregates was determined by hydrogen peroxide oxidation of mSOC. As the SOC mineralization was low in $<53 \mu\text{m}$ fraction as detailed above, it could also be assumed that the mineralization of mSOC of macroaggregates and microaggregates would also be slow. So, the evidence from the current work reinforced the idea that the adsorption of SOC on silt-plus-clay particles (i.e. physico-chemical protection) offers stabilization against microbial decomposition and chemical oxidation. Moreover, the estimated MRT of stable SOC of macroaggregates and microaggregates using radiocarbon measurements showed that MRT of

stable pool could vary 1100-2400 years (Chapter 7). Therefore, the physico-chemical protection might offer longer stabilization of SOC.

8.1.4.2. Chemical composition influences physico-chemical stabilization of SOC

The chemical composition of SOM was determined in Chapter 4. The content of O-alkyl carbon decreased with increased alkyl carbon content in $<53 \mu\text{m}$ fractions and microaggregates compared to macroaggregates. The SOM fractions that were isolated from macroaggregates and microaggregates showed a decrease in O-alkyl carbon but increase in alkyl carbon content from LF to mSOC. The selective accumulation of alkyl carbon might increase MRT_s and hence reduce SOC mineralization in $<53 \mu\text{m}$ fraction due to hydrophobicity of alkyl carbon. Bachmann et al. (2008) stated that higher hydrophobicity stabilize SOC by reducing water, oxygen and nutrient availability to microbes. The mSOC in macroaggregates and microaggregates was enriched with alkyl carbon (27% of total NMR signal intensity) which might be the reason for higher stabilization of mSOC against hydrogen peroxide oxidation (Leifeld and Kögel-Knabner, 2001) and microbial decomposition. Therefore, chemical composition might influence the stabilization of SOC.

8.1.4.3. Physical protection influences chemical composition of SOC

The fPOC content of microaggregates was 64% higher than that of macroaggregates but the BSR and MRT_s of both aggregates were similar (Chapter 2 and 3). Six et al. (2002a, 2002b) stated that fPOC is physically protected inside macroaggregates and microaggregates but decomposition of fPOC still continues at slow rate. The decomposition may continue until the labile carbon (e.g. carbohydrates, proteins) of fPOC is used up by the microbes (Golchin et al., 1998). In Chapter 5 pore geometry of macroaggregates and microaggregates was evaluated. The porosity and pore connectivity of microaggregates was lower than that of macroaggregates. However, the estimated pore size distribution of macroaggregates and microaggregates using X-ray microtomography (μCT) indicated that the diameter of smallest detectable pore was $5.2 \mu\text{m}$. It is important to note that the smallest pore diameter estimated by μCT is highly dependent on the resolution of scanning. So, the aggregates may contain pores $<5.2 \mu\text{m}$ that remained undetected due to the limit set by resolution of scanning. van der Linden (1989) showed that pores $<0.2 \mu\text{m}$ in diameter are not accessible to bacteria. Fungal hyphae are generally $1-15 \mu\text{m}$ in diameter and mycorrhizal hyphae are just few micrometers across (Hudson, 1992). So, the μCT observed pores in microaggregates were accessible to both bacteria and fungi. The O-alkyl carbon content in macroaggregates and

microaggregates was high (44-57%) and as the μ CT observed pores were accessible to microbes, decomposition of O-alkyl carbon rich fPOC might be responsible for similar BSR and MRT_s of both aggregates.

The physical protection of SOC does not involve only inaccessibility of microbes but also includes slow SOC decomposition due to poor aeration (von Lützow et al., 2006). In chapter 5 it was observed that the pore space connectivity of macroaggregates was higher than that of microaggregates. Moreover, macroaggregates contained large pore space 100-250 μ m in diameter range, which may provide flow paths of gases and nutrients to the interior of macroaggregates. But in both aggregates pore size distribution was dominated by <40 μ m pores which can create intermittent anoxic condition in microaggregate and certain portion of macroaggregates (Zausig et al., 1993). The macroaggregates isolated by wet sieving in the current study also contained microaggregates (bound-microaggregates). So, high heterogeneity in water and oxygen distribution inside macroaggregates and microaggregates could slow SOC decomposition. In Chapter 4 it was observed that alkyl carbon accumulation was higher in microaggregates than macroaggregates. Buurman and Rosecoe (2011) stated that the accumulation of recalcitrant compounds occurs in soil when the conditions of decomposition are not favorable for microbes. Moreover, Stevenson (1994) reported that in soil lipid decomposition is slow under anaerobic condition. Therefore, the occlusion of SOC in microaggregates (both free and bound) has the potential to slow down the microbial decomposition due to unfavorable environmental conditions and to induce the accumulation of alkyl carbon concentration in microaggregates.

8.1.5. Relative importance of different SOC stabilization mechanisms under different land uses

A lower aggregate associated SOC in both macroaggregates and microaggregates under crop-pasture rotation compared to native pasture and woodland was observed in the current study. The lower SOC content in aggregates in crop-pasture rotation compared to native pasture and woodland could result if decomposition rate of SOC was faster due to aggregate breakdown. Lower aggregate-SOC content under crop-pasture rotation could also be due to smaller carbon input compared to native pasture and woodland. However, Six et al. (2000) suggested that the faster turnover of macroaggregates is considered dominant process of SOC loss under convention tillage. Although the studied crop-pasture rotation was not as intensively tilled as conventional tillage, the moderate disturbance might also increase the breakdown of macroaggregates which was supported by relatively higher water stable

microaggregate content under crop-pasture rotation compared to native pasture and woodland (Chapter 2). On average, the fPOC content was also lower in crop-pasture rotation which also indicated that soil management had significant influence on aggregate breakdown and release of fPOC from aggregates. The relatively high alkyl/O-alkyl carbon ratio in microaggregates and <53 μm fraction also suggested that SOC under crop-pasture rotation was more decomposed than native pasture.

The estimated MRT_s under crop-pasture rotation and woodland were longer than that of native pasture (Chapter 3). This conclusion was supported by the longer MRT under woodland and crop-pasture rotation in the current study measured by radiocarbon technique (Chapter 7). Oades (1988) stated that cultivation preferentially removes younger SOC from soil aggregates and increases the relative concentration of older SOC. So, the relative increase in older carbon in aggregates under crop-pasture rotation could account for the longer MRT. The longer MRT of SOC under woodland can be explained by the absence of soil disturbance and higher aryl carbon content in woodland litters. In chapter 4 it was observed that woodland LF and cPOC had very high aryl carbon content compared to native pasture and crop-pasture rotation which evidently represented high aromatic carbon content in woodland litters.

Unlike fPOC content as stated above mSOC was not affected by land use which was evidently due to higher stabilization of adsorbed SOC. Therefore, physico-chemical protection might be of greater significance for SOC stabilization under crop-pasture rotation whereas in native pasture and woodland physical and physico-chemical stabilization of SOC were important. It is important to note that biochemical recalcitrance also appears to influence SOC stabilization in woodland.

In conclusion, adsorption of SOC on the silt-and-clay fraction (mSOC) could stabilize SOC against microbial decomposition and chemical oxidation. Moreover, the fPOC became physically protected due to low pore connectivity and accumulation of alkyl carbon in microaggregates. Land use determined the relative importance of stabilization mechanisms in soil aggregates. The physico-chemical protection of SOC was of higher importance under crop-pasture rotation. So, SOC in microaggregates was stabilized mainly by physico-chemical protection mechanism. Low pore connectivity rather than inaccessibility was principal factor of physical protection of SOC in microaggregates.

8.2. Soil organic matter stabilization in Oxisols

8.2.1. Key findings

A hierarchical arrangement of aggregates was found in the studied Oxisols. On average, the cPOC and fPOC comprised about 15% and 8% of aggregate-SOC. The mSOC stored on average 64% and 67% SOC in macroaggregates and microaggregates, respectively. Cropping led to lower SOC concentrations in all aggregate size fractions, which confirmed that SOC decomposition in cropping was faster than in improved pasture and forest due to deterioration of soil aggregates. The basal respiration rate did not vary between macroaggregates, microaggregates and <53 μm fractions. Moreover, the effect of land use on basal respiration rate was not significant. The aggregate porosity and pore connectivity were higher in microaggregates. The influence of land use did not significantly alter aggregate pore geometry (Chapter 6).

8.2.2. Synthesis

The findings of the current study suggest that in Oxisols adsorption of SOC and Fe/Al oxides on silt-plus-clay particles were responsible for microaggregates formation. The slow decomposition of fPOC in macroaggregates and microaggregates could also enhance SOC stabilization. The decomposition of fPOC might alter the pore geometry of aggregates. The physico-chemical protection was most significant mechanism of SOC stabilization in microaggregates. These findings are elaborated in the following sections.

8.2.3. Microaggregate formation and SOC stabilization mechanism

The mSOC represented 66% of SOC in Oxisols which suggested cementation of soil aggregates by SOC could be an important aggregate formation mechanism. The Oxisols pedogenically have high sesquioxides concentrations in the soil matrix (Beinroth et al., 2000) resulting in high SOC sorption capacity (Kaiser and Guggenberger, 2006; Mikutta et al., 2006). Shang and Tiessen (1998) reported 50% of SOC of Oxisols and Alfisols was associated with the silt fraction and microaggregate stability of these soils was dependent on the cementation by organic matter, kaolinite and oxides of Fe and Al. Wagai and Mayer (2007) stated that organic matter-Fe-oxides-clay association is important for both microaggregate formation and stabilization. In Chapter 6 it was been demonstrated that the percent water stable macroaggregate content of Oxisols had a significant positive correlation with macroaggregate mSOC ($r = 0.44$, $p < 0.01$), suggesting that the interaction of mSOC with

sesquioxides and clay might influence microaggregate formation and the stabilization of SOC.

In Chapter 6 it was shown that fPOC represents on average 8% of aggregates-SOC of both macroaggregates and microaggregates, respectively. The fPOC contents in both macroaggregates and microaggregates of Oxisols were low compared to Alfisols in which fPOC content varied between 8-30% of aggregate-SOC (Chapter 2). The measurements of radiocarbon signature in aggregates also showed that SOC in Oxisols was older than that of Alfisols (Chapter 7). This suggests that in Oxisols, fPOC in both aggregates was decomposed to a greater extent than in Alfisols and that the decomposition products were adsorbed on the silt-plus-clay particles (mSOC). A negative correlation between fPOC content in microaggregates and its mSOC which was nearly significant ($r = -0.46$, $p=0.056$) supports the proposition that decomposition products of fPOC might influence the mSOC content.

It has been shown in Chapter 6 that the porosity and pore connectivity of microaggregates were higher than that of macroaggregates. The pore size distribution showed that the $>40 \mu\text{m}$ pores occupied up to 4% of macroaggregates and 3% of microaggregates. In both aggregates total porosity was dominated by $<40 \mu\text{m}$ pores. The 3D images of Oxisol macroaggregates showed that, similar to Alfisols microaggregates occur inside macroaggregates (Fig. 8.2). But, unlike Alfisol microaggregates the Oxisol microaggregates had more connected pore volume and contained $<125 \mu\text{m}$ stable microaggregates inside the scanned microaggregates of 250-125 μm in diameter. The $<125 \mu\text{m}$ microaggregates could be cemented by sesquioxides and organic matter to form microaggregates of 250-125 μm (Oades and Waters, 1991). The organic matter (e.g. fPOC) in $<125 \mu\text{m}$ microaggregates might be more decomposed compared to macroaggregates. Therefore, the higher porosity in microaggregates than macroaggregates could be due to formation of pores after complete decomposition of fPOC. Oades and Waters (1991) stated that complete decomposition of POC could form pores in aggregates. So, the fPOC could be occluded macroaggregates and microaggregates but slow decomposition of fPOC and adsorption of fPOC decomposition products on silt-clay-particles could enhance SOC stabilization and aggregate formation through interaction of SOC with sesquioxides and clay particles.

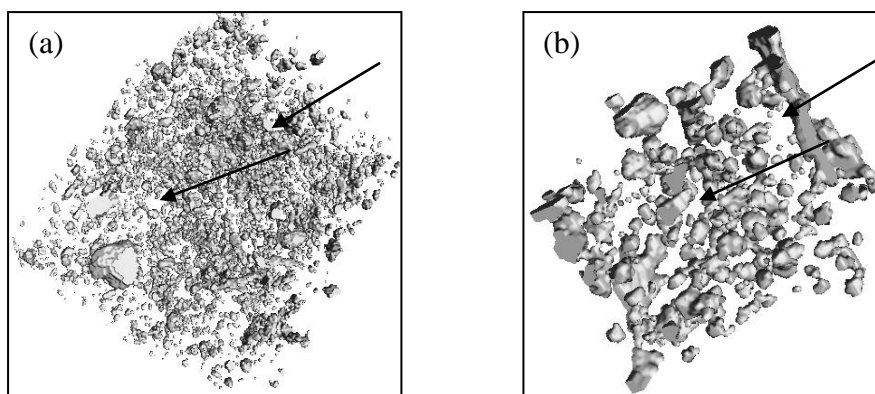


Fig. 8.2. Example of 3D images of macroaggregate (a) and microaggregate (b) of Oxisol showing pores in grey and soil matrix in white. The black arrows indicate the presence of microaggregates

8.2.4. Relative importance of different SOC stabilization mechanisms under different land uses

A significantly lower aggregate associated SOC and MWD were found in both macroaggregates and microaggregates under cropping compared to improved pasture and forest (Chapter 6). The lower SOC in aggregates under cropping could result from SOC turnover rate that was faster due to rapid breakdown of aggregates (Section 8.1.5). The higher MRT of SOC in both macroaggregates and microaggregates indicated that cultivation preferentially removes younger SOC from soil aggregates. The mSOC content was lower in cropping compared to improved pasture and forest. However, under cropping the mSOC represented on average 62% of aggregate associated SOC. Therefore, the physico-chemical protection might be of higher significance for SOC stabilization under cropping.

In summary, (i) SOC appeared to contribute to microaggregates formation (ii) the complete decomposition of POC occluded in microaggregates could account for pore spaces creation (iv) physico-chemical protection was most significant mechanism of SOC stabilization in microaggregates.

8.3. Conclusion and future implications

Integration of a range of techniques in the current study led to the major conclusion that physico-chemical protection was the dominant SOC stabilization mechanism in both Alfisols and Oxisols that were studied. Physical protection in microaggregates also played an important role in SOC stabilization in the Alfisols. Crop-pasture rotation and cropping had a

profound influence on physically protected SOC in microaggregates and under these land uses physico-chemical protection was important to stabilize SOC.

It is also important to translate the laboratory microscale findings on SOC stabilization to the field scale because under field conditions, *in situ* soil structural units are identifiable and pore networks around these units potentially have a pronounced effect on SOC stabilization. The experimental evidences of SOC stabilization mechanisms that presented in this current work would suggest that it is important to devise a comprehensive SOC fractionation scheme from soil aggregates taking soil structural condition under field into consideration. More detailed work is needed to understand the effect of the pore geometry of microaggregates on microbial function and SOC stabilization. Some of the future research directions are listed below:

- i) As the physical protection, MRT of SOC in both macro and microaggregates was similar; a more simplified SOC fractionation scheme can be developed. Briefly, un-complexed organic matter from >2 mm aggregates can be separated by slaking and subsequent density flotation which can be termed as cPOC. The remaining heavy fraction could be separated into occluded POC (oPOC) and mSOC by complete dispersion of soil. The field observed >2 mm aggregates could be used for the SOC fractionation to better reflect the field condition and to eliminate the effect of grinding and drying on SOC fractions.
- ii) The estimation of microbial biomass and diversity in both macroaggregates and microaggregates and its relationship with pore geometry of aggregates. However, a need for development of macroaggregates and microaggregate isolation technique still exists by which it would be possible to control mixing up microbes and dissolved SOC during isolating microaggregates from macroaggregates.
- iii) The concentration of SOC in nano-pores needs to be investigated. Using both X-ray μ CT images of pore geometry and images of SOC distribution in aggregates might help to resolve the effect of pore geometry on SOC storage.
- iv) It is also necessary to confirm the relative contribution of plant derived and microbe derived carbon in mSOC as this fraction is regarded important for long term SOC stabilization.

v) Solid state ^{13}C NMR provides a general overview of SOC present in soil. However, pre-treatment with HF could possibly modify the SOC composition which needs a critical investigation. Research can be directed towards better identification of functional groups of SOC e.g. carbohydrate and lignin by ^{13}C NMR.

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