Can Fe oxides stabilise organic carbon in soil?

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by

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Abstract

Soil aggregates are known to play an important role in preserving soil organic carbon (SOC). Poorly crystalline Fe oxides are one of the main aggregating agents in tropical soils. The interaction between soil organic matter (OM) and Fe oxide surface leads to the formation of an organo-mineral complex which is one of the main mechanisms of SOC preservation. The effect of Fe oxide amendment on soil aggregate formation as well as the preservation of SOC in temperate soils is largely unknown. Therefore, the main objective of this research project is to investigate these effects through a number of laboratory studies.

Soils were amended with different levels of Fe oxides (0%, 0.5, 1%, 2% and 4%). An initial adsorption study confirmed that Fe oxide amendment can adsorb dissolved organic carbon (DOC) and significantly reduce the concentration of DOC in five different soil types ($P \leq 0.05$). This result gave a justification for further plant growth experiment for 8 weeks. Soils for the plant growth experiment were amended with the same levels of Fe oxides, organic matter (OM) (0 t/ha, 1.5 t/ha, 3 t/ha, 6 t/ha and 12 t/ha), and a combination of these different levels of Fe oxides + OM. After 8 weeks, Fe oxide amendment caused an increase in soil macro-aggregates followed by a significant increase in SOC in macro-aggregate fractions (($P \leq 0.05$). In addition, the masses of resistant soil organic carbon (rSOC) fractions and SOC concentration in rSOC fractions significantly increased due to Fe oxide amendment ($P \leq 0.05$). The effects of OM and Fe oxide+ OM were only significant on SOC concentration in macro-aggregates.

These results showed that Fe oxide amendment can certainly be a useful tool to stabilise SOC through the formation of stable soil aggregates which could mitigate the impacts of global warming.

Keywords: Soil aggregates, organic carbon, Fe oxides, adsorption.

Table of contents

List of Tables

(Short titles)

List of figures

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Declaration

I declare that the work presented in this thesis is original and completely my own work. This work has not previously been submitted for any other degree or award at this, or any other University. All sources are acknowledged as References.

Chapter 1

General Introduction

1.1. Introduction

The significant increase in atmospheric CO_2 since industrial revolution has given rise to a global concern regarding the survival of the Earth's interconnected ecosystems (Lal, 2004). The total estimated global emission of CO_2 due to fossil fuel combustion is 270±30 Pg (Pg = petagram = 10^{15}g = 1 billion tons) and 136±55 Pg due to deforestation, conversion of forest to agricultural land, changes in cultivation, and mismanagement of lands (Lal, 2004). Soils are widely recognised as one of the largest terrestrial pools of atmospheric carbon (Lal, 1997; Follet, 2001; Lal, 2004). They contain approximately 1500 gigatons (1 gigaton = 1 billion tons) of organic carbon within the first 1 m of the surface horizon (Abdullahi et al., 2018). Besides being an active medium for plant growth, maintaining water quality, and a wide range of biodiversity, soils have the potential to mitigate climate change through the sequestration of organic carbon (IPCC, 1996; Kane, 2015; Lal, 2004). In contrast, CO_2 can be released back to the atmosphere through mineralisation of soil organic matter (Kirschbaum, 2000; Six et al., 1998). Therefore, increasing soil organic carbon (SOC) sequestration as well as minimising mineralisation of organic carbon have been subjected to great research interest.

The magnitude of SOC release and stock is largely dependent on the interaction between soil structure and soil organic matter (SOM) (Blanco-Canqui et al., 2004). SOM is the most complex component in soil comprising of a heterogeneous mixture of organic substances that originate from the plant materials, microbial by-products, and animal bodies with varying stages of decomposition (Christensen, 1992). Soil structure is an organisation of soil aggregates holding numerous pore spaces within the aggregates and between the aggregates (Tisdall, 1996). Aggregates are formed by the arrangement of sand, silt and clay particles where several organic and inorganic compounds act as a cementing agent to bind these particles together (Tisdall, 1996). Interestingly, SOM and soil structure are two interdependent factors. SOM influences the structural development of soil by mediating numerous biotic functions, water retention and soil fertility (Beare et al., 1994a). In return, stable soil structure protects mineralisation of SOM (Anger and Chenu, 2018) by providing physical, chemical, or biochemical stabilisation of SOM inside the soil aggregates (Verchot et al., 2011; Tisdall and Oades, 1982; Six et al., 2002). The role of soil aggregates in preserving SOC has been acknowledged in many studies (Tisdall and Oades, 1982; Six et al., 2002; Blanco-Canqui et al., 2004). The most direct evidence is that the disruption of soil aggregates causes increased mineralisation of SOM compared to undisrupted aggregates (Gregorich et al., 1989; Rovira and Greacen, 1957; Gupta and Germida, 1988). Agricultural soils are subjected to frequent disruption of aggregates due to intensive farming practices, low organic matter input, and lack of sustainable management (Greenland, 1977; Six et al., 1998). Increasing the formation of stable aggregates in arable land could be one of the major options in order to tackle global carbon emission (Smith et al., 1997). The mechanisms of soil aggregate formation and the ability of soil aggregates to stabilise SOM are largely dependent on soil biotic, abiotic and climatic factors (Ramesh et al., 2019; Blanco-Canqui and Lal, 2004). Research suggests that SOM acts as a dominant agent for aggregate formation in moderately weathered temperate soil whereas Fe or Al oxides are the main aggregate forming agents in tropical soils (Six et al., 2002). Tropical soils show better aggregate stability than temperate soils due to the presence of oxides and 1:1 type clay minerals (Six et al., 2002). Organic materials such as humic compounds are the main agents for the formation of stable micro-aggregates whereas fungal hyphae, bacteria, plant roots, polysaccharides are the main agents for macro-aggregate formation (Denef and Six, 2005). Fe oxides increase soil aggregate formation and stabilise soil organic carbon through adsorption and complexation reaction with soil constituents (Muggler et al., 1999; Oades et al., 1989; Poras et al., 2017). The surface characteristics of Fe oxides are the most influential factors that affect their ability to increase soil aggregates (Muggler et al., 1999). Due to a larger surface area and the more reactive surface of poorly crystalline Fe oxides, it has a greater effect on soil aggregate formation compared to the crystalline Fe oxides (Duiker et al., 2003). Knowledge regarding the effect of Fe oxides on soil aggregate formation and SOC stabilisation in temperate soil is limited.

Therefore, the main objectives of this thesis were to investigate the individual effect as well as the interactive effect of poorly crystalline Fe oxides and organic matter on increasing soil aggregates and stable organic carbon in an arable cultivable land under temperate climate. This was achieved through a number of laboratory experiments (Chapter 2, Section 2.11.)

1.2. Thesis outline

This thesis comprises six chapters including this general introduction as the 1st Chapter. The contents of each chapter are briefly described below:

- Chapter 2 reviews the available previous studies on how soil aggregates form, factors affecting soil aggregation, mechanisms of soil organic carbon stabilisation, and the role of Fe oxides on stabilising soil organic carbon. It also provides current knowledge on different methods of soil organic carbon fractionation.
- Chapter 3 represents all the methodologies used to run the laboratory experiments in Chapter 4 and Chapter 5.
- Chapter 4 investigates the effect of Fe oxides on reducing the release of organic carbon from different soil types. The experiment was carried out to observe whether Fe oxide can adsorb dissolved organic carbon (dissolved organic carbon is one of the most available forms of organic carbon in soil) or not. It provides a justification for running a further experiment to see the effect of Fe oxide on stabilising organic carbon in soil aggregates.
- Chapter 5 investigates the effect of Fe oxides on soil aggregate formation and stabilising soil organic carbon in aggregate fractions. A pot experiment was conducted for 8 weeks. After 8 weeks, the masses of different aggregate fractions were measured and compared with the masses of aggregate fractions before 8 weeks. Based on significant changes in masses of aggregate fractions by the 8 week time, the concentration of soil organic carbon was measured in the aggregate fractions.
- Chapter 6 gives a general conclusion by providing information on the key findings of this thesis and the scope for further research based on the current findings.

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Chapter 2

A review: Aggregate formation and the role of aggregation in preserving soil organic carbon

2.1. Introduction

Soil aggregates are the structural units of soil that contribute to soil fertility and quality. They are produced by the rearrangement, flocculation, and cementation of primary soil particles (sand, silt and clay) through the combined activity of organic and inorganic compounds in soil (Amezketa, 1999; Bronick and Lal, 2005; and Plaza-Bonilla, 2013). Aggregates are mainly categorised as micro-aggregates (diameter ≤ 0.25 mm) and macro-aggregates (diameter > 0.25 mm) based on their size. Soil aggregates are known to play an important role in enhancing soil biological activity (Hattori, 1988), nutrient sorption (Wang et al., 2001), water storage (Prove et al., 1990), gaseous exchange ((Lynch and Bragg, 1985) and organic carbon storage (Tisdall and Oades, 1982). The amount of organic matter preserved in soil represents one of the largest reservoirs of organic C on a global scale (Schlesinger, 1995). Therefore, the mechanism of carbon stabilisation in soil has received much research interest with an aim to manage the atmospheric C sink. The role of soil aggregates in stabilising soil organic carbon (SOC) is a key element in soil C dynamics. This chapter reviews the current knowledge regarding soil aggregation, different factors controlling aggregation and the mechanisms by which soil aggregates are thought to stabilise SOC with particular reference to the role of Fe oxide on SOC stabilisation. In addition, several fractionation methods for determining the partitioning of C between different fractions, and their applicability are reviewed.

2.2. Aggregate formation

Aggregation results from the action of numerous natural agencies that help to cluster soil particles together (Martin et al., 1955). According to the aggregate hierarchy theory (Tisdall and Oades, 1982), aggregates are sequentially formed (Figure 2-1). Micro-aggregates are formed first as the building blocks for the formation of macro-aggregates. Three different types of binding agents (transient, temporary and persistent) are responsible for aggregation at different hierarchical stages (Tisdall and Oades, 1982). Transient binding agents are microbial and plant-derived polysaccharides, produced through the degradation of plant and animal residues. These are produced rapidly but are highly susceptible to microbial decomposition and degradation by changes in soil management and last for days to a few weeks. Temporary binding agents consist of roots, root hair and fungal hyphae, particularly vesicular-arbuscular mycorrhizal hyphae that last for months to several years. Persistent binding agents are mainly aromatic humic substances, amorphous alumina-silicates, Fe and Al oxides, polysaccharides associated with di and trivalent metal cations, resistant fragments of organic matter and microbial cells that are independent of management practices.

According to the hierarchal theory, free primary particles and silt-sized aggregates (<20 µm) are bound together by persistent binding agents and form water-stable micro-aggregates (20–250 µm) which are resistant to degradation by soil management. Then the temporary and transient binding agents bind the stable micro-aggregates together to form macro-aggregates (>250 µm) which can be disrupted by crop rotation, tillage and other cultivation practices. Aggregate hierarchy theory was slightly modified by Oades (1984) (Figure 2-1). He proposed that roots and hyphae bind macro-aggregates together and form a nucleus for micro-aggregate formation in the centre of macro-aggregates. The decomposed fragments of roots and hyphae are combined with microbially produced mucilages and encrusted with clays to initiate micro-aggregate formation within macro-aggregates.

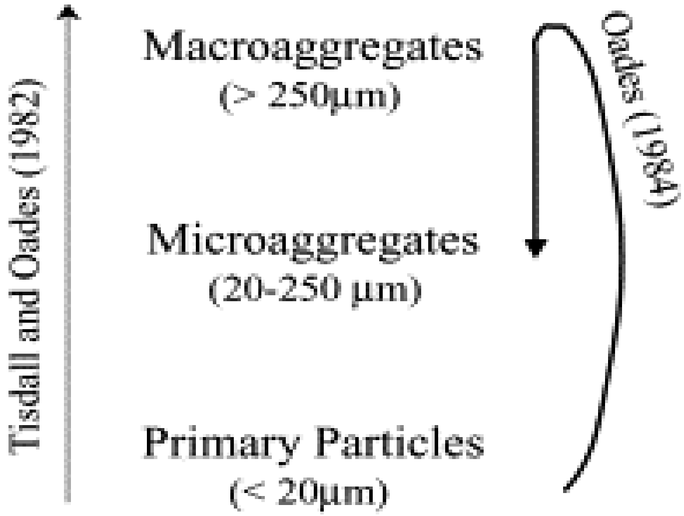

Figure 2- 1: Formation of aggregate hierarchal orders described by Tisdall and Oades (1982) vs. advanced by Oades, (1984) (taken from Six et al., 2004).

2.3. Aggregate stability

The ability of soil aggregates to withstand stresses caused by water immersion, wind erosion, or other land management practices is known as aggregate stability (Papadopoulos, 2009). The extent of aggregate disruption upon wetting depends on two fundamental processes: slaking and dispersion (Rengasamy et al., 1984).

Water reduces the cohesion of soil particles and causes a subsequent reduction in aggregate stability. When the disruptive forces of water are stronger than the forces by which soil particles are bound together in the aggregates, slaking results (Collis-George and Lal, 1971; Emerson, 1977). The susceptibility of soil aggregates to slaking can be reduced by the presence of inorganic and organic binding agents (Russel, 1971) such as iron oxide, aluminium oxides, calcium carbonate and organic materials. These binding agents strengthen the bonds within aggregates and prevent slaking (Quirk and Panabokke, 1962).

When aggregates are immersed in water, clay dispersion results from the swelling of clay particles to such an extent that the attractive forces between the particles are not strong enough to hold them together (Emerson, 1977). In soil suspension, cations associated with clay surfaces are held by electrostatic forces. The difference in electric potential between the bulk soil solution and the solution at the particle interface is called zeta potential. Dispersed clay particles in soil solution repel each other under high zeta potential. The presence of electrolytes (flocculating agents, mainly Ca^{2+}, Mg^{2+}, and K^{+}) lower the zeta potential and reduce the repulsion of dispersed clay particles which help particles to mutually attract each other and settle as a floccule (Gedroits, 1955, Sargent, 2015). Stable aggregates are formed when these flocculated particles are bound together by organic or inorganic binding agents (Gedroits, 1955).

2.4. Factors affecting soil aggregate formation

Soil aggregation, as a complex dynamic process results from the continuous interaction of soil physical, chemical, and biological agents (Ray, 1998). Tisdal and Oades, (1982) suggested that physical forces involved in the process of wetting and drying, compression by roots, and organo-mineral interaction are mainly responsible for soil aggregation. In addition, some of the chemical processes in combination with the effect of soil organic matter also play an important role in soil aggregation (Pullemen et al., 2005). Excrements produced by animals and plant root exudates, act as a glue to hold soil particles together and increase aggregate formation (Silva de Neto et al., 2016) particularly within the surface horizons of many soils (Harris et al., 1966). Factors controlling soil aggregation can be divided into three broad categories: climatic, biotic and abiotic factors.

2.4.1. Climatic factors

Climate influences soil aggregation through variation in temperature and moisture regimes, resulting in varying wet-dry and freeze-thaw cycles, which cause a reorientation of soil particles (Singer et al., 1992) and affect soil aggregation. (Six et al., 2004).

2.4.1.1. Freeze-thaw cycle

Freezing can cause aggregate formation (Lehrsch, 1998) and degradation (Chepil, 1954; Sillanpää and Webber, 1961; Edwards, 1991). According to Lehrsch et al. (1993) when a soil aggregate is affected by frost, only the wetter part of the aggregate becomes susceptible to disruption and there is always a drier part just below the ice lens. In the wetter part, ice crystals expand into the pores and break the particle-to-particle bonds. In contrast, drying helps to create a closer contact of particles by shrinking soil mass or precipitating binding agents.

2.4.1.2. Alternate wetting and drying

Soils are continuously subjected to cycles of wetting and drying through the action of wind, rainfall, and sun. Wetting and drying cycles help aggregate formation in compacted arable non-aggregated soil, by breaking up soil clods and releasing smaller particles to form new aggregates (Chaney and Swift, 1986), particularly micro-aggregates (Dorioz et al., 1993). In well-aggregated soil wetting causes slaking and clay dispersion (Denef et al., 2001). Drying facilitates an intermolecular association between the organic molecules and mineral particles in the soil, resulting in increased aggregate stability (Kemper and Rosenau, 1984). Soils that contain a higher amount of organic matter shows higher aggregate stability upon wetting and drying compared to that of soil with lower organic matter content (Hayens and Swift, 1990). The reason behind this is that soil with high organic matter shows a reduced wettability due to the presence of hydrophobic components in the organic materials and also creates many intermolecular associations with mineral surfaces upon drying (Caron and Angers, 1996). Soil mineralogy influences the effect of wetting and drying cycle on aggregation. Aggregates in soils dominated by kaolinite clays or non-swelling clays are twice as stable as swelling clays upon wetting (Burroughs et al., 1992).

2.4.2. Abiotic factors

2.4.2.1 Soil types

There are different mechanisms of aggregation in different soil types (Table 2-1). Soil properties such as CEC (cation exchange capacity), the surface area of clay minerals and organic matter content affect aggregate formation and aggregate stability. Aggregation is dominated by the action of cations in soil with low clay concentration and SOC but the action of cations is minimal in soil with high SOC and clay concentration. For example, in Oxisol and Ultisol non-crystalline Al^{3+} or Fe^{3+} hydroxides are the main aggregating agents that stabilise soil organic carbon through the formation of Fe/Al–humus complexes that protect SOC from microbial decomposition (Oades and Waters, 1991). Carbonates are the predominant factors causing high aggregate stability in Aridisols (Boix-Fayos et al., 2001).

Table 2- 1 Soil types and aggregation factors (taken from Bronick and Lal, 2005).

Soil orders	Aggregation factors	Authors
Alfisols	SOM	Dalal and Bridge, 1996, Oades and Waters, 1991
Andisols	Allophane clay, non-crystalline clay	Torn et al., 1997
Aridisols	Carbonates and weathered clay minerals	Boix-Fayos et al., 1998, Boettinger and Southard, 1995
Entisols	SOM	Dalal and Bridge, 1996
Inceptisols	Amorphous clay	Dalal and Bridge, 1996
Oxisols	Al^{3+} and Fe^{3+} oxides	Oades and Waters, 1991, Dalal and Bridge, 1996
	Non-crystalline Al hydroxides	
	Plant roots and rhizosphere	
	Hydrophobic SOM	
Spodosols	Organo-metallic complexes	Deconinck, 1980
	Metallic hydroxides	
Ultisols	SOM	Dalal and Bridge, 1996, Zhang and Horn, 2001
	Non-crystalline sesquioxides	
Vertisols	Clay-size fractions	Leinweber et al., 1999, Dalal and Bridge, 1996
	Polycationic bridges, wet-dry cycles	

2.4.2.2 Texture

Research suggests that finer-textured soil has greater aggregate stability than heavy textured soil (Mamedov et al., 2007). The presence of clay particles has a predominant role over silt and sand particles on the aggregate formation (Mamedov et al., 2007). Clay particles act as a cementing agent that holds particles together to form aggregates (Emerson, 1977).

2.4.2.3. Cation exchange capacity (CEC)

Exchangeable cations in soils contribute to the aggregate formation (Dimoyiannis et al., 1998). Polyvalent cations in soil (Ca^{2+}, Al^{3+}, and Fe^{3+}) form cationic bridges between negatively charged clay content and organic matter, reducing the repulsive forces between the clay particles and OM, which results in increased aggregate formation (Tisdall, 1996).

2.4.2.4. Soil pH

Clay particles are susceptible to flocculation in soil with high pH (Haynes and Naidu, 1998), which helps to join particles together to form aggregates. Boix-Fayos et al. (2001) suggested that large aggregates are formed in soil with a high pH. Sometimes, lime is added to acidic soil to enhance the microbial activity, crop yield, and SOM content which in turn increases aggregation (Haynes and Naidu, 1998).

2.4.2.5 Binding Agents for aggregation

Soil Organic Matter (SOM)

SOM is one of the most important contributory factors for aggregation that has been intensively studied in the literature (Angers, 1998; Chaney and Swift, 1984; Bronick and Lal, 2005; Kemper and Koch 1966; Six et al., 2004). SOM contains a variety of humic (polysaccharides, humic acid, fulvic acid) and non-humic (protein, lignin, carbohydrates) substances that are known to increase aggregate formation because of their gluing properties (Calabi Floody et al., 2011). Colloidal humic substances in organic matter bind with negatively charged clay minerals through their polar groups such as COOH, OH, NH_2 and form clay-humus complex in soil (Myers, 1937). This process increases aggregate formation in soil. Adsorption of humic substances on clay particles is another mechanism of aggregate formation in soil (Evans et al, 1959). The effect of SOM on aggregation was reviewed by Tisdall and Oades (1982).

They made some important points:

- A part of freshly derived organic components like mono- or poly-saccharides, and living organic components like roots and fungal hyphae stabilises soil aggregates.
- Above a critical concentration, soil organic matter has no further effect on aggregation (but they did not mention what the concentration is)
- The disposition of soil organic matter is more important than the type or amount of organic matter.
- Particle orientation is more important for aggregate stability than the contribution of SOM.

Anger and Giroux (1996) suggested that recently added particulate organic matter (POM) initiates aggregation. Fresh POM acts as a substrate for fungi and bacteria and they bind the soil particles through encrustation with mucilage or physical enmeshment. Aggregates of 100-200 µm often contain plant debris in their core and in these cases, the main mechanism of aggregation is the encrustation of plant fragments with mineral particles (Oades and Waters, 1991). Golchin et al. (1994) proposed the mechanism of micro-aggregate (20-250 µm) formation by organic residues. They suggested that when POM enters the soil, microbes colonise it rapidly. The excretory by-products of these microbes are strongly adhesive and adhere to soil mineral particles which consequently encrust the organic fragments. If SOM contains a substantial amount of hydrophobic compounds, it reduces the susceptibility of aggregates to slacking by producing non-uniform hydrophobic coatings on the aggregate surfaces (Mbagwu and Piccolo, 1989; Haynes and Swift, 1990; Sullivan, 1990; Zhang and Hartge, 1992)

Clay mineralogy

Clay minerals are the most reactive minerals in soil because of their high specific surface area and surface charge. They are involved in the interaction with ions and organic matter in soil, resulting in the formation of organo-mineral assemblages which is crucial for soil aggregate formation (Angers, 1998; Six et al., 2000a). Soils usually contain a mixture of several clay minerals depending on the degree of weathering and the parent materials (Wilson,1999). Temperate soils contain a mixture of layer silicates such as illite, chlorite, and kaolinite (Robert et al., 1991; Wilson, 1999) with a predominance of phyllosilicates in most agricultural soil (Virto et al., 2008). Research suggests that there is a strong positive correlation between organic carbon content and clay mineral in soil (Schimel et al., 1985; Wiseman and Püttmann, 2006). Clay minerals with higher CEC and specific surface areas generally improve aggregate formation than those of lower CEC and specific surface area (Six et al., 2000; Amezketa, 1999). Research suggests that kaolinitic minerals have high flocculation capacity and their association with iron oxides shows strong aggregate stability and resistance to slaking (Amezketa, 1999, Denef et al., 2002). At lower organic matter level, the presence of oxides and variable charge clay minerals cause higher aggregation whereas at higher organic matter levels the presence of mixed clay minerals causes higher aggregation. Clay mineralogy can be responsible for aggregate breakdown by slaking and chemical dispersion. According to Emerson (1964), swelling clays are less likely to breakdown by slaking than non-swelling clays as the pressure of entrapped air inside the clay particle is diffused by swelling. Illites are the most sensitive clay minerals to chemical dispersion due to their higher flocculation value, smaller edge to phase attraction and irregular surface (El-Swaify, 1976; Oster et al., 1980; Shainberg and Letey, 1984). The influence of clay minerals on aggregation is actually varied by other soil properties like soil organic matter or texture (Denef and Six, 2005; Norton et al., 2006; Reichert et al., 2009).

2.4.3. Biotic factors

2.4.3.1. Roots

There are five mechanisms through which roots affect soil aggregation: (1) root penetration (2) changed soil water regime (3) root exudation (4) dead root decomposition and (5) root entanglement (Angers and Caron, 1998; Degens, 1997).

Root penetration increases the breakdown of large water-stable macro-aggregates by 20 -50% (Materechera et al., 1994; Monroe and Kladivko, 1987). In contrast, roots help to increase aggregation by changing the soil moisture regime. There is a saturated water film along with the roots so that water flows along with them (Six et al., 2004). Continuous uptake of soil water through roots creates a dry region in the vicinity of the roots which facilitates the binding of root exudates on clay particles (Reid and Goss, 1982). Root exudates reduce the slaking of aggregates by increasing pore tortuosity and decreasing the wetting rate of aggregates (Caron and Angers, 1996). Dead root decomposition causes greater microbial activities in the top layer of soil by providing a supply of energy and nutrients. Therefore, the amount of microbial metabolite production increases. These microbial metabolites act as an adhesive to bind soil particles together and increase aggregate formation. Root entanglement promotes the stabilisation of macro-aggregates (Tisdall and Oades, 1982; Miller and Jastrow, 1990; Jastrow et al., 1998). In leguminous plants, rhizospheres act as a habitat for a large population of microorganisms that contribute to aggregation and SOC stabilisation. Therefore, leguminous plant roots facilitate higher microbial biomass and higher soil aggregating capacity compared to non-leguminous plants. (Chan and Heenan, 1996; Haynes and Beare, 1997).

2.4.3.2. Microorganisms

Microbes decompose soil organic materials and produce enzymes and extracellular polymers as their metabolic by-products which have a significant positive effect on soil aggregation. Enzymes mineralise high molecular weight compounds in soil (Kandeler and Murer, 1993) while polysaccharides act as a glue to bind soil particles together which are the key mechanisms for micro-aggregation (Chenu, 1993; Baldock et al., 1990). Fungal spores and mycelium are known to increase aggregate stability (Harris et al., 1964). Tisdall and Oades (1979) reported that fungal hyphae entangle soil particles or micro-aggregates together which are further glued together by microbial polysaccharides, resulting in the formation of macro-aggregates. Aggregation increases with the density of fungal hyphae (Haynes and Beare, 1997). Arbuscular mycorrhizal fungi (AMF) are one of the most dominant fungi responsible for aggregation (Jastrow and Miller, 1998). They produce glomalin (a kind of glycoprotein that has a high concentration and recalcitrant in nature) which increases aggregate stability (Wright and Upadhyaya, 1998; Rillig et al., 2002a.)

2.4.3.3. Soil fauna

Soil fauna increases soil aeration, porosity, infiltration, litter mixing, nutrient cycling, metal mobility, C turnover, N, and C stabilisation, which are all directly or indirectly involved in soil aggregation (Six et al., 2004; Brown et al., 2000). Among all other organisms, the impact of earthworms is widely acknowledged in many studies.

Earthworms are known to improve soil aggregation through several biological and physicochemical processes (Brown et al., 2000). They mix organic materials with soil particles or other inorganic components in the soil through their burrowing activity and ingest the mixture (Martin, 1991). This mixture passes through their gut and is excreted as cast. During gut transit, the soil microstructure is completely destroyed, and a restructuring of the soil and organic matter occurs, resulting in the formation of numerous micro-aggregates inside the casts (Six et al., 2004; Shipitalo and Protz, 1988). Earthworm casts contain microbial polysaccharide and other organic compounds that strengthen the bond between soil particles and other mineral components, resulting in the formation of stable soil aggregates (Shipitalo and Protz, 1988). Martin (1991) found that SOM in earthworm casts are protected from microbial decomposition for a long time. However, the influence of earthworms on aggregation varies with the species of earthworms (Winsome and McColl, 1998). There are three main ecological groups of earthworms: epigeic, anecic, or endogeic (Bouché, 1977). Epigeic and anecic earthworms have relatively little or no effect on aggregation compared to endogeic earthworms (Shipitalo and Bayon, 2004; Binet and Curmi, 1992). Endogeic earthworm species directly improve soil aggregation through their extensive burrowing activity in the upper 10-15 cm of soil surface. They form a sub-horizontal network of burrows in search of their food and ingest a mixture of soil and organic matter. They excrete this mixture as earthworm cast which contains numerous micro-aggregates (Shipitalo and Bayon, 2004).

2.5. Mechanisms of soil organic carbon stabilisation in soil aggregates

Soil organic carbon (SOC) exists in a thermodynamically unstable state (Schmidt et al., 2011). Decomposition of organic materials such as dead roots, plant residues in agricultural soil takes place within a few days to years, resulting in a rapid release of CO_2 to the atmosphere (Wardle et al., 1999). Several environmental and biological factors enable these thermodynamically unstable organic materials to preserve in soils for decades to centuries (Schmidt et al., 2011). Previous studies suggested three main mechanisms of SOC stabilisation: Chemical, physical, and biochemical (Christensen, 1996; Stevenson, 1994, Six et al., 2002).

2.5.1. Chemical stabilisation

Chemical stabilisation of SOC involves the formation of organo-mineral complexes by silt and clay particles that protect organic carbon content from decomposition (Six et al., 2002; Hassink, 1997; Sorensen, 1972). The formation of organo-mineral complex is largely dependent on the chemical characteristics of mineral fractions in soil (Baldock and Skjemstad, 2000). Clay mineral fractions with high specific surface area and surface charge density stabilise SOC by providing available adsorption sites for the organic matters. Hassink (1997) investigated the relationship between soil texture and carbon stabilisation. He found that there is a strong relationship between SOC stability and silt plus clay particles whereas there is no correlation between texture and the amount of carbon in sand-sized fractions. Organic carbon stability in a chemically protected organo-mineral complex increases with an increase in silt plus clay content (Guggenberger et al., 1999; Sorensen, 1972). The presence of $CaCO_3$, amorphous Fe or Al oxide is known to increase the amount of stable organic carbon in soil by producing Ca-organic, Al-organic or Fe–organic linkage (Baldock et al, 2000).

2.5.2. Physical Stabilisation

Protection of SOC inside soil aggregates is referred to as physical stabilisation. Three main mechanisms for physical stabilisation are found in the literature. (1) the compartmentalisation of substrate and microbial biomass (Killham et al., 1993;) (2) formation of anaerobic condition inside the micro-aggregates and reduced oxygen diffusion inside macro-aggregates (Sexstone et al., 1985) which inhibits microbial activity within the aggregates (Sollins et al., 1996) and (3) the compartmentalisation of microbial biomass and microbial grazers (Elliott et al., 1980).

According to Hattori (1988), microbial abundance is significantly higher in the outer part of aggregates than the inner part, and aggregates hold organic carbon at their centre (Six et al., 2002; Golchin et al., 1994). Therefore, soil aggregates act as a compartment to protect SOC from microbial decomposition. Golchin et al. (1994) suggested a compositional difference between free and occluded light fractions of organic carbon within the aggregates. The occluded light fraction of SOC contains higher amounts of recalcitrant C compounds such as fatty acids, lipids, cutin acids, proteins, peptides and lower amounts of easily decomposable carbohydrate and polysaccharide than the free light fraction. Cultivation or aggregate turnover causes the free light fractions of SOC to enter into the intra-aggregate pore, then the easily decomposable portion of it becomes accessible to microbes and the recalcitrant portion of it becomes preserved within the aggregates. These findings suggest that aggregate turnover promotes rapid mineralisation of SOC and a substantial loss of easily decomposable carbon. Six et al. (2000b) suggested that fine particulate organic carbon can be stabilised inside the micro-aggregates within macro-aggregates or in free micro-aggregates under no-tillage. Several studies suggest that micro-aggregates stabilise SOC better than macro-aggregates, therefore incorporation of C into micro-aggregates enhances the long-term protection of SOC (Six et al., 2002; Skjemstad et al., 1996). The maximum amount of SOC can be stabilised by maximising the formation of micro-aggregates in soil (Six et al. 2000).

2.5.3 Biochemical stabilisation

Biochemical stabilisation of SOC takes place because of the complex chemical composition of soil organic carbon. This complex composition can be the original composition of organic matter such as the composition of plant or animal residues or the altered composition of organic matter through decomposition, condensation and complexation of decomposed organic materials which render the organic matter recalcitrant and inaccessible to microbes (Six et al., 2002).

2.6. Role of Fe oxides on stabilising SOC

The role of Fe oxides in increasing soil aggregation and SOC storage has been studied mainly in tropical soils (Kleber et al., 2005; Wei et al., 2016; Duiker et al., 2003; Honghai et al., 2008). There are three main mechanisms through which Fe oxide can improve soil aggregate formation and stabilise soil organic carbon: (a) adsorption of organic matter on Fe oxide surface (Oades et al., 1989) (b) electrostatic bonding between the positively charged oxide surface and the negatively charged clay surface (El-Swaify and Emerson, 1975) (c) Cationic bridging: positively charged Fe oxide form a bridge between negatively charged clay mineral surface and organic matter (Edwards and Bremner,1967).

Mechanisms involve in adsorption of organic matter on the Fe oxide surface are ligand exchange-surface complexation, hydrogen bond, van der Waals forces, and hydrophobic forces (Honghai et al., 2008; Kalbitz et al., 2000). Physical adsorption through hydrophobic force was recognised as the predominant mechanism of dissolved organic carbon adsorption by Fe oxide (Jardine et al., 1989). However, many studies suggested that the formation of organo-mineral complex through ligand exchange is the dominant mechanism of dissolved organic matter adsorption on the Fe oxide surface (Kalbitz et al., 2000; Gu et al., 1994; Honghai et al., 2008).

Electrostatic interaction between positively charged Fe oxide and negatively charged clay particles causes clay particles to flocculate and reduce clay dispersion, therefore increasing micro-aggregation which plays an important role in SOC stabilisation (Schofield and Samson, 1953).

Cationic bridging by Fe oxide between clay minerals and organic matter leads to the formation of clay-polyvalent cation-organic matter complex which is a dominant mechanism of SOC stabilisation (Edwards and Bremner, 1967).

In addition, some other studies suggest that Fe-(hydr) oxides can precipitate on clay mineral surface, this coating of Fe-(hydr) oxides increases the specific surface area of clay minerals and provides available sorption sites for organic matter (Goldberg, 1989; Arias et al., 1995).

The interaction between Fe oxide and kaolinite mineral is found as one of the dominant mechanisms of SOC sequestration in many studies (Arias et al., 1995; Kitagawa, 1983; Wiseman and Püttmann, 2006). A comparative study by Saidy et al. (2012) showed that the interaction of Kaolinite minerals with non-crystalline Fe oxide causes a greater reduction in the amount of SOC mineralisation than that of smectite and illite.

2.6.1. Factors affecting the role of Fe oxides on SOC stabilisation

There are both positive and negative pieces of evidence regarding the effect of iron oxides on soil aggregate formation and SOC stabilisation (Oades and waters, 1991; Greenland et al., 1968). Several factors are supporting this variable behaviour of Fe oxides. The variability in soil characteristics (type of soil, type of organic matter, pH, and ionic composition of soil solution) and the crystallinity of Fe oxides control the capacity of Fe oxides to stabilise organic carbon in soil aggregates (Duiker et al., 2003; Goldberg, 1989).

Type of soil

Highly weathered soils in tropical and subtropical regions contain a substantial amount of sesquioxide (oxides of Fe or Al). Peng et al. (2015) found that Fe oxides act as a major micro-aggregating agent in Ultisol. Fe oxide has a dominant effect on SOC sequestration in Oxisol in tropical regions because of their higher quantity compared to Luvisol and Mollisol in the temperate regions (Six et al., 2002).

Type of organic matter

According to Gu et al. (1995), high molecular weight organic compounds are preferentially adsorbed by Fe oxide compared to the low molecular weight compounds. The adsorption capacity of Fe oxide increases with the presence of aromatic ring, N- and S-containing group, amino acid, carboxylic acid group in organic molecules (McKnight et al., 1992).

Ionic composition of soil solution

Fe oxides react with organic anion in soil solution and form an organo-metal complex which is an important mechanism of SOC stabilisation in soil (McLean and Bledsoe 1992). Several studies suggest that anions in soil solutions such as sulphate and phosphate compete with dissolved organic carbon for the adsorption site (Tipping, 1981; Gu et al., 1995; Kalbitz et al., 2000). DOC shows a greater affinity for the adsorption site than sulphate (Kaiser and Zech, 1998a) in forest soils.

Soil pH

Adsorption of dissolved organic carbon on Fe oxide surface is known to occur at the acidic condition in forest soils (Kalbitz et al., 2000; Goldberg 1989; Honghai et al., 2008). Soil pH significantly affects the adsorption capacity of Fe oxide by changing the surface functional group of Fe oxide and ionisation of dissolve organic carbon (Honghai et al., 2008). At pH value below the point of zero charge (PZC) of Fe oxide mineral, hydroxyl group of Fe oxide mineral become protonated which results in an increase in net positive charge. Organic acidic group become 100% ionised when pH is two units above the ionisation constant (pKa) of organic acid, depending on the number of acidic group presents per molecule (Bowden et al., 1973, 1974). Therefore, Adsorption of organic acidic group on Fe oxide surface is the greatest when soil solution pH range is below the PZC of Fe oxide and above the pKa of organic acid (Murphy et al., 1990a,b). Kaiser (1996) suggested that the ability of Fe oxide to adsorb dissolved organic carbon decreases significantly if the soil pH is above 6.5 and below 4.5. Honghai et al. (2008) suggested that adsorption of organic matter through ligand exchange takes at pH less than 7.5, above this pH adsorption may still take place by Vander Waals force. Porras et al. (2017) studied the effect of Fe and Al oxide on SOC sequestration within the pH range 3.9-4.9. They found that the solubility of metal oxides increases with decreasing pH and the amount of stable SOC concentration was the greatest at the lowest pH.

Crystallinity of Fe oxide

Research suggests that the surface characteristics of Fe oxides are the most influential factors that affect their ability to increase soil aggregates. Due to a larger surface area and more reactive surfaces of poorly crystalline Fe oxides, it has a greater effect on soil aggregate formation compared to the crystalline Fe oxides (Duiker et al., 2003).

2.7. Relationship between SOC stability and aggregate turnover

Soil aggregate turnover directly affects SOC stabilisation. Physical disturbance in agricultural soils such as tillage causes macro-aggregate turnover, thereby preventing the formation of micro-aggregates inside the macro-aggregates. Six et al. (1998) developed a conceptual model (Figure 2-2) explaining the influence of aggregate turnover on SOC stabilisation rates. Their model showed that SOC stabilisation rate decreases with an increase in aggregate turnover. The model describes the role of particulate organic matter dynamics on aggregate formation and degradation. In general, soil microbes decompose fresh plant residues and produce mucilage which acts as a binding agent to form macro-aggregates around the coarse intra-aggregate POM (iPOM) (>250µm). Coarse iPOM further breaks down into fine iPOM (53–250 µm) by microbial processes. Fine iPOM encrusted with soil mineral surface by microbial mucilage. This mineral encrusted fine iPOM act as a stable organic core of a new micro-aggregate within the macro-aggregates. Macro-aggregate turnover causes the release of coarse iPOM and expose fine iPOM to further microbial decomposition, resulting in a decrease in formation of new micro-aggregates and the physical protection of soil organic carbon inside the micro-aggregates (Six et al., 2000a).

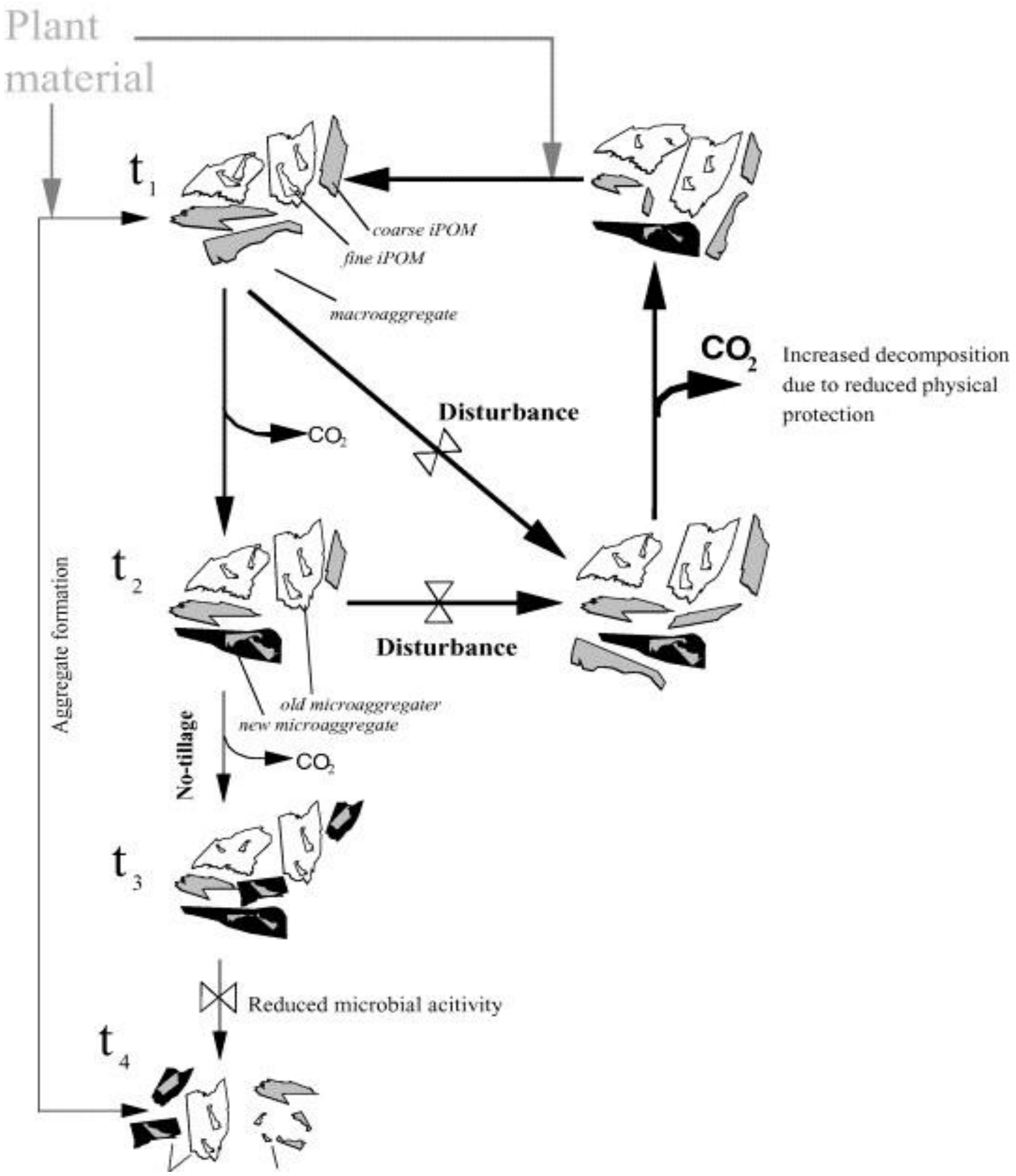

Figure 2- 2: This conceptual model showing the formation of new micro-aggregates within macro-aggregates and the stabilisation vs mineralisation of organic carbon under different levels of physical disturbance (eg. No-tillage, tillage) in agricultural soils. (Taken from Six et al., 2000a).

2.8. Fractionation of SOC

In order to characterise the reactivity of SOC within natural soil environment, it has been categorised into a variety of different pools (Table 2-2). These pools indicate the stability of SOC against biological decay, decomposition rate, and their turnover time (Stockmann et al., 2013). Several factors including chemical composition of soil organic matter, association with clay minerals and oxides as well as its location within the soil influence the access of microbes to SOC (Sollins et al., 1996; von Lützow et al., 2006). Most commonly used SOC fractionation methods are either physical (size, density, aggregation) or chemical (extraction, hydrolysis, and oxidation).

Table 2- 2: SOC pools based on their decomposition rate (Taken from Baldock, 2007).

Pool category	Decomposition rate (half-life)	Forms of SOC	Composition
Fast or labile	days to years	Surface plant residue	leaf litter and crop/pasture material
Fast or labile	days to years	Buried plant residue	greater than 2 mm in size residing within the soil
Fast or labile	days to years	Particulate organic matter	Semi-decomposed organic material smaller than 2 mm and greater than 50 μm in size
Slow or stable	Years to decades	Humus	Well decomposed organic material smaller than 50 μm in size that is associated with soil particles
Passive or recalcitrant	Decades to thousands of years	Resistant organic carbon	Charcoal or charred materials that results from the burning of organic matter (resistant to biological decomposition)

2.8.1. Physical fractionation

SOC separation by physical fractionation reflects the physical protection of organic carbon within aggregates by preventing the accessibility of SOC to decomposer organisms (Balesdent, 1996, von Lützow et al., 2007). The association of SOC with soil matrix and the distribution of particulate organic matter between and within the soil aggregates can be determined by physical fractionation method (Golchin et al., 1994; Beare, 1994, Six et al., 1998,). Three different fractionation processes are mainly referred to as physical fractionation. These are: i) aggregate size fractionation (undispersed soils <2mm are wet-sieved) ii) particle size fractionation (soils are dispersed before wet-sieving), and iii) density fractionation (subdivided into light fraction and heavy fraction).

2.8.1.1. Aggregate size fractionation (without preceding dispersion of soil)

Aggregate size fractionation of SOC involves the separation of free organic carbon, organic carbon occluded within macro and micro-aggregates and in clay microstructure (Von Lützow et al., 2007). In this method, undispersed soil <2mm are wet sieved and sedimented. Wide ranges of aggregate size classes are obtained. Aggregates <20 µm are very stable and can be separated from micro-aggregates (<20-250 µm) by using ultrasonic treatment (Oades and Waters, 1991). However, many nanometres to micrometre sized micro-aggregates are present within clay sized aggregates (<2 µm) which cannot be separated by ultrasonic treatment (Chenu and Plante, 2006). In temperate regions most aggregates do no break down into primary particles upon immersion into water but rather into small stable units of micro-aggregates. Therefore, aggregate size fractionation for temperate soils indicates the hierarchy of aggregates (Oades and Waters, 1991; Tisdall and Oades, 1982).

2.8.1.2 Particle size fractionation (preceding dispersion of soil)

The association of SOC with silt and clay-sized particles leads to the formation of organo-mineral complex which is a crucial mechanism for the stabilisation of SOC (Six et al., 2002b, Guggenberger and Kaiser, 2003). Von Lützow et al. (2007) showed the possible distributions of SOM across particle size fractions in temperate soils (Figure 2-3), and the corresponding distributions of SOM quality and turnover rates. They showed that the gradual increase in SOM content is associated with decreasing particle size. However, SOM turnover time varies in different particle-size fractions, (von Lützow et al., 2007). The most common methods that fractionate SOC according to particle size use sieving and sedimentation of dispersed soil (dispersion is carried out by ultrasonic vibration or a chemical dispersant) (Christensen, 1992). Particulate organic matter (POM) is separated from coarse (approximately>50 µm fractions) particle size fractions by sieving and sedimentation (Cambardella and Elliott, 1993)

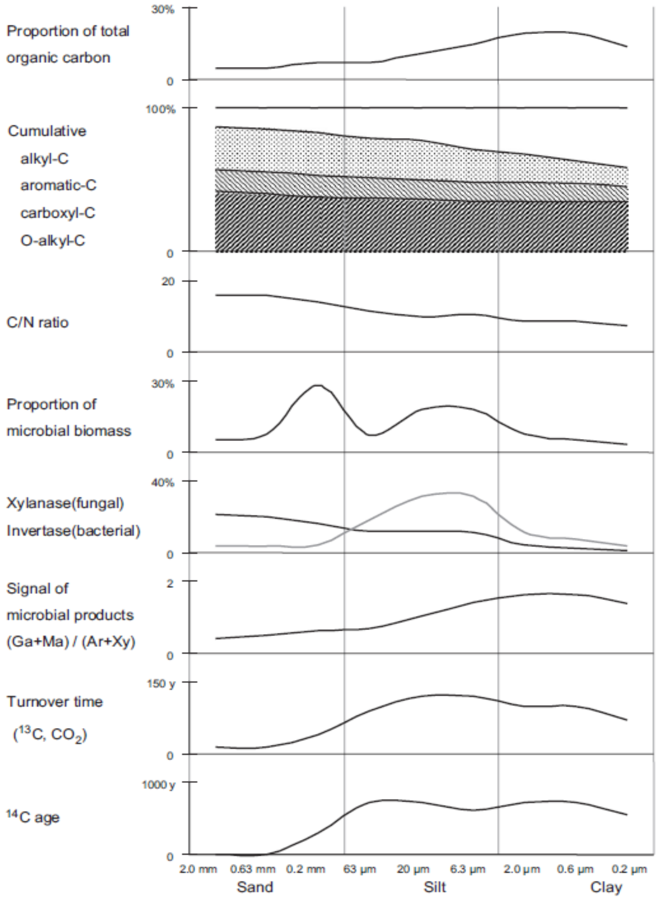

Figure 2- 3: Typical distributions of SOM across particle size fractions in temperate soils and the corresponding distributions of measures of SOM quality and turnover rates (von Lützow et al., 2007)

2.8.1.3. Density fractionation (preceding dispersion of soil)

Density fractionation is used to separate soil organic carbon which is not firmly bound with soil minerals. It separates SOC into two fractions: heavy and light. It is generally carried out by submersion of soil samples into inorganic salt solutions with a specific density, typically 1.6 to 2.2 g cm^{-3} (Christensen, 1992). The association of SOC with phyllosilicate is identified (von Lützow et al., 2007) by density >1.6–2 g cm^{-3}. The lighter fractions that have a density <

1.6–2 g cm^{-3} contain mainly free or occluded particulate organic matter (Christensen, 1992). Various degrees of dispersion are used prior to density fractionation in order to breakdown the organo-mineral bonds within aggregates. This allows the separation of various sized free SOM and organo-mineral complexed SOM.

2.8.2 Chemical fractionation

Chemical fractionation separates organic carbon into various components based on their solubility, and chemical reactivity in a variety of extracting agents, as well as their resistance to different oxidizing agents (von Lützow et al. 2007). There are three different means of chemical fractionation: extraction, hydrolysis and oxidation.

2.8.2.1 Extraction

Extraction involves the removal of organic substances from an inorganic soil matrix such as sand, silt and clay. Then the carbon content of the extractant is analysed. Different extractants are used depending on the nature of organic matter to be separated. Repeated extraction is used for the maximum recovery of organic carbon from soil. Cold water or aqueous solutions of different ionic strengths are used for the extraction of the bioavailable fraction of organic carbon (<45 µm in size) such as organic acids, phenols, and carbohydrates (von Lützow et al., 2007). Microbial biomass carbon can be separated by chloroform fumigation (Jenkinson, 1976; Vance et al., 1987). Furthermore, alkaline solutions (most commonly 0.1M NaOH) are used for the extraction of humic materials from soil (Stevenson, 1994). Humic acid fractions are soluble in alkali but insoluble in acid whereas fulvic acid fractions are soluble in both alkali and acid. Nonpolar compounds like fatty acid, lipid, long chain alcohol, waxes, resins, are extracted by organic solvents such as n-hexane, chloroform, dichloromethane/ methanol (Schnitzer and Schuppli, 1989, Naafs et al., 2004).

2.8.2.2 Hydrolysis

Hydrolysis removes potentially biodegradable compounds such as proteins, nucleic acids or polysaccharides, and leaves behind a recalcitrant fraction of bio-macromolecules. In general, hydrolysis of SOC is carried out with hot water or with acids. Hot water extracts the readily decomposable carbon pool from total soil organic carbon (Henriksen and Breland, 1999; Sparling et al., 1998). Acid hydrolysis disrupts hydrolytic bonds between aggregates and releases carbohydrate and protein materials, leaving biologically recalcitrant alkyl material intact (Leavitt et al., 1996; Martel and Paul, 1974). 2% HCl is used to hydrolyse hemicellulose while 80% H_2SO_4 is used to hydrolyse crystalline cellulose (Waksman, 1936). Polyvalent cations involved in micro-aggregation can be removed by acid hydrolysis which makes the occluded or complex SOC soluble. Therefore, acid hydrolysis mobilises SOC stabilised by occlusion in micro-aggregates, sorbed by polyvalent cation bridges and complexed SOC (Oades, 1988).

2.8.2.3 Oxidation

Oxidation is a fractionation process where oxidising reagents ($KMnO_4$, H_2O_2, NaOCl, and $Na_2S_2O_8$) are applied to mimic strong enzymatic degradation of SOC to an extent that oxidation preferentially removes less protected SOC and the residual product after oxidation contains more intimately associated SOC with mineral surfaces (Kaiser and Guggenberger, 2007, von Lützow et al., 2007). SOC resistant to chemical oxidation is resistant to microbial decomposition and has the slowest turnover time (Balesdent, 1996).

A brief description of SOC fractionation methods with their possible advantages and disadvantages are presented in Table 2-3.

Table 2- 3: A brief description of soil organic carbon fractionation methods

Fractionation method	SOC fractionated from	Treatment	Size/nature of separated fraction	(+)Advantages/ (-) disadvantages of the method	References
Physical fractionation					
Aggregate size	Undispersed soil <2mm in size	1) Wet sieving and sedimentation	1) Free organic carbon 2) SOC occluded within macro (>250 µm) or micro aggregates (20-250 µm)	(+) Useful as a pre-treatment to obtain more homogeneous fractions (-) do not consist of the functional fractions needed for modelling.	Von Lutzow et al., 2007
	Micro-aggregates <20-250 µm	2) Ultrasonic treatment	SOC occluded within clay microstructure (<20 µm) aggregates		Oades and waters, 1991
Particle size	Dispersed soil <2mm	**Step1:** Dispersion by using any of the following 1) Ultrasonic vibration 2) Shaking 3) Chemical dispersant **Step2:** Wet or dry sieving sedimentation	1) Coarse particulate organic matter >250 µm 2) Micro-aggregates 250-53 µm 3) Silt+clay fraction <53 µm	(+) Provides a rough differentiation between young (active) and older (intermediate and passive) SOM (-) Not homogeneous in terms of SOC turnover time and cannot be equivalent to model pools.	Balesdent et al., 1998; Cambardella and Elliot, 1993; Christensen, 1992
Density	Dispersed soil <2mm	**Step1**: Dispersion by using any of the following 1) Ultrasonic vibration 2) Shaking 3) Chemical dispersant **Step 2**: Submersed into inorganic salt solution (most commonly sodium-poly tungstate, silica gel) with a density 1.6-2 gcm^{-3} **Step3**: Sieving and flotation	1) Heavy fraction (mineral associated fraction; density > 2 gcm^{-3}) 2) Light Fraction (labile fraction) • Free light fraction = <0.6 gcm^{-3} • Occluded light fraction (oLF) I=1.6-1 gcm^{-3} • (oLF) II=1.6-1.8 gcm^{-3} • (oLF) III =1.8-2.0 gcm^{-3}	(+) more finely distinguished fractions (-) Only quantity characterisation	Christensen, 1992; Gregorich and Janzen, 1996; Rovira and Vallejo, 2003

Chemical Fractionation					
Extraction	Undispersed soil <2mm in size	Extrication by using Cold or hot water or ionic solution	Dissolve organic carbon (DOC) <0.45 µm	(+) determine the level of organic matter distributed in water solution (+) easy performance (-) only quantity characterisation	Thurman, 1985
		1) **Step1**: 24 hours Chloroform fumigation **Step2**: extraction with 0.5 M K_2SO_4 for 2 h (shaking)	Microbial biomass carbon (MBC)	(+) smart concept for determination of micro-organism amount (–) no determination of enzymes activity	Vance et al.1987; Jenkinson,1976
		2) 0.1M NaOH +0.1M $Na_4P_2O_7$ Or HCl	1)Dissolve organic carbon (DOC) of humic or nonhumic origin 2) DOC complexed with metal or clay (by $Na_4P_2O_7$ extraction)	(-) Na^+ ions interfere with the flocculation of clays, causing disaggregation. (+) $Na_4P_2O_7$ extracts 'complexed OM'	Alexandrova , 1960; Stevenson, 1994
		3) Organic solvent (n-hexane, chloroform, dichloromethane/methanol)	• Fatty acid (n hexane extractable) • Long chain alcohols, and wax esters (chloroform extractable) • Lipid (dichloro-methane or methanol extractable)	(+) isolate the recalcitrant SOC fraction (-) Compound specific isotopic analysis is required to understand stabilisation mechanism of SOC	Schnitzer and Schuppli,1989; Naafs et al., 2004; Wiesenberg et al., 2004
Hydrolysis	Undispersed soil <2mm in size	Hydrolysis with 1) Hot water (60 min. gentle boiling in distilled water)	carbohydrates, amino-N, and amides	(+) easy performance (-) only quantity characterisation	Leinweber et al., 1995
		2) H_2SO_4 **Step1**: 2.5 M H_2SO_4 (30 min. at 105 °C) **Step 2**: 13 M H_2SO_4 (20 °C overnight, next dilution with 1M H_2SO_4, 3 h at 105 °C)	• Labile Pool I (LP I): (hydrolysed by 2.5 M H_2SO_4) • Labile Pool II (LPII): (hydrolysed by13 M H_2SO_4 +1M H_2SO_4) – LP I	(+) both quality and quantity characterisation (+) very sensitively distinguish fractions (+) correlated to RothC model (+) suitable for various substrates	Rovira and Vallejo, 2000; Shirato and Yokozawa, 2006

			• Recalcitrant Pool: (Total organic carbon – LPII)		
		3) HCl **Step1**: 1M HCl **Step 2**: 6M HCl	Labile SOC pool Cellulose and hemicellulose fraction	(-) less suitable for organic substrates	Silveira et al., 2008
Oxidation	Undispersed soil <2mm in size	Oxidation with 1) (33mM) $KMnO_4$	• Fraction 1: Labile C fraction (glycol group, sugar, amino acid) • Fraction 2: Total organic carbon – Fraction 1	(+) easy performance (-) only quantity characterisation	Tirol-padre and Ladha, 2004
	<20 µm particle	2)H_2O_2	labile C fraction (removed 90% SOC)	(+) Suitable tool to isolate functionally passive pool (-) Less dispersive effect on clay micro-aggregates	Leifeld and Kögel-Knabner, 2001; Theng et al., 1992; Von Lutzow et al., 2007
	Mineral soil fraction (density >1.6 g cm^{-3})	3)NaOCl	Humic materials 77% and 95% mineral associated SOC	(+) Allows mineralogical analysis of clays	Guggenberger and Kaiser, 2003
	Clay mineral and Fe oxides associated SOC	4)$Na_2S_2O_8$	16-99% mineral associated SOC	(+) characterise SOC pool stabilised by interactions with the mineral matrix (-) allow only a qualitative analysis	Eusterhues et al., 2003; Kiem and Kögel-Knabner, 2002; Meier and Menegatti, 1997

2.9. Combined SOC fractionation methods

Individual fractionation schemes are unlikely to reflect the multiple mechanisms of SOC stabilisation. Several studies have suggested that the best approach for SOC fractionation is a combination of both physical and chemical fractionation schemes (Sohi et al., 2001; Six et al., 2002; Zimmermann et al., 2007a). Stable isotopic measurement and spectroscopic methods could be a powerful tool for SOC separation. Estimation of highly resistant SOC may be obtained by combining multiple measurements in sequential schemes. For example, a combination of size fractionation and a density or chemical fractionation can be used to isolate highly refractory SOC. Cristopher et al. (2018) suggested the feasibility of 20 different SOC fractionation methods for temperate agricultural soil (Table 2-4). They found a very good overall performance index (O.P.I) for the fractionation methods used by Sanderman et al. (2014), Balesdent, (1987), Six et al. (2000) and Zimmermann et al. (2007a, 2007b). Sanderman et al. (2014) used only particle size fractionation method followed by NMR spectroscopy in order to separate the resistant soil organic carbon within three measured SOC fractions and examine the typical understanding of carbon flow. They followed the RothC modelling (Rothamsted carbon model shown in Figure 2-4) of organic carbon pool and hypothesised a conceptual model containing three organic C pool similar to RothC. They concluded that RothC model was a poor representation of carbon flux through their soil. Balesdent (1987) investigated SOM turnover by using radiocarbon dating. He fractionated SOC as coarse and fine fraction by using the combination of particles and density fractionation. He used both $Na_4P_2O_7$ and NaOH extractant at the same pH and showed that organic carbon obtained by $Na_4P_2O_7$ was older than organic matter extracted by NaOH. However, the humin fraction found in his study was heterogeneous and a logical analysis behind the heterogeneity was not given. Six et al. (2000) separated five different fractions of SOC ranging from macro-aggregates, micro-aggregates within macro-aggregates, intra-macro-aggregates, mineral fraction, and free micro-aggregates in order to estimate the contribution of micro-aggregates for carbon sequestration under no-tillage condition. They suggested a slower macro-aggregate turnover to sequester new carbon within the micro-aggregates. The relative reactivity of each organic carbon fraction and the mechanism of C sequestration within the micro-aggregates were not clearly described. Later, Six et al. (2004) proposed a conceptual model of soil organic matter dynamics with four SOC pools, demonstrating three main mechanisms of SOC sequestration within soil aggregates. They concluded that there might be an overlap in stabilisation mechanism of SOC between different pools. Zimmermann et al. (2007b) used an alternative measure of SOC fractionation by following the conceptual SOC pools of the RothC model and investigated the relationship between their measured fractions and the corresponding fractions of the RothC model. They found that a strong correlation coefficient between the quantified SOC fractions and RothC modelled pools under various site-specific conditions. They suggested that in order to find a fractionation procedure similar to RothC pools their proposed method can be used with minimum modification.

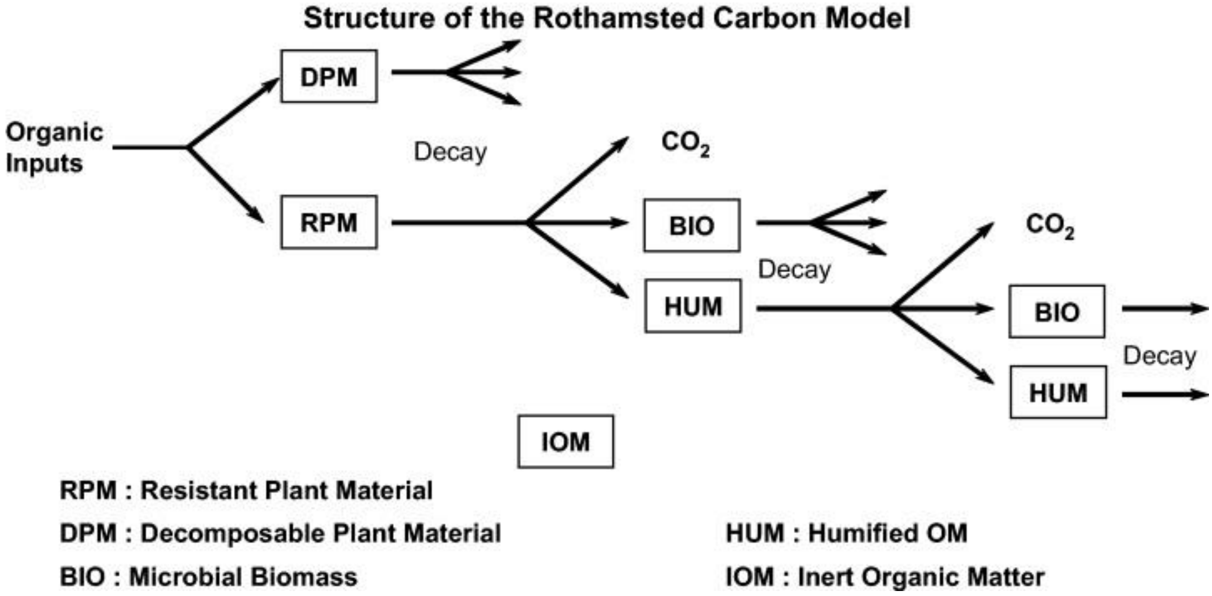

Figure 2- 4: Soil organic carbon pools defined by RothC (taken from Zimmermann et al., 2007b).

Table 2- 4: Feasibility of 20 different SOC fractionation methods for temperate agricultural soil, including fractionation methods applied, with class and type of method, original reference, number of fractions isolated, dispersion method, applied density [gcm^{-3}], chemicals used for oxidation/extraction/hydrolysis (Ox/Ex/Hyd), size ranges of the isolated particles or aggregates [μm] and the overall performance index (O.P.I) for each method (taken from Cristopher et al., 2018).

ID	**Class**	**Type**	**Reference**	**n**	**dispersion**	**Density**	**Ox/ex/hyd**	**Size fraction**	**O.P.I**
Agg1	Physical	Aggregates	Elliot, 1986	3				0 < 53<250 < 2000	Good
Agg2		Aggregates	Six et al., 2000a	3				0 < 53<250 < 2000	Good
Par1		Particle	Sanderman et al., 2014	2	HMP			0 < 50>2000	Very good
Par2		Particle	Lopez-Sangil and Rovira, 2013 a	4	Ultrasonic			0 < 20<50< 200<2000	Good
Den1		Density	Sollins et al., 1984	2		1.6			Fair
Den2		Density	Sollins et al., 2009	5	Ultrasonic	1.6,2,2.4,2.8			Good
Den3		Density	Golchin et al., 1994a, Golchin et al., 1994b	3	Ultrasonic	1.6			Fair
Agg +Den		Aggregates + Density	Six et al., 1998	10	HMP	1.85		0 < 53<250 < 2000	Good
Par+ Den1		Particles + Density	Six et al., 1998 a	4	HMP Glass bleads	1.85		0 < 53<2000	Fair

Par +Den2		Particles + Density	Shaymukhametov et al., 1984	5	Glass bleads	2		0 < 2 < 50 < 250 < 1000	Fair
Par +Den3		Particles + Density	Diochon et al., 2016	5	Glass bleads	1.7		0 <5<53 <250<2000	Good
Par+Den4		Particles + Density	Steffens et al., 2009 a	5	Ultrasonic	1.8		0< 20<2000	Fair
Par+Den5		Particles + Density	Balesdent, 1987	5	Glass bleads	1		0 < 50<200 < 2000	Very good
Che1	Chemical	Oxidation	Mikutta et al., 2006	2			NaOCl, HA		Good
Che2		Hydrolysis	Rovira et al., 2012 a	4			H_2SO_4		Very good
Com1	Combined	Particles + Extraction		5	Glass bleads		K_2SO_4	0 <63<2000	Good
Com2		Particles + Oxidation	Leifeld and Kögel-Knabner, 2001	3	ultrasonic		H_2O_2	0 < 20	Good
Com3		Aggregates + Oxidation	Six et al., 2000	7	HMP		NaOCl	0 < 53<250 < 2000	Very good
Com4		Particles + Density + Oxidation	Zimmermann et al., 2007a,b	5	ultrasonic	1.8	NaOCl	0 < 0.45<53 < 2000	Very good
Com5		Aggregates + Particles + Density + Extraction	Kaiser et al., 2016 a	10	Ultrasonic	1	$Na_4P_2O_7$	0 < 0.45<53 < 250	Good

HMP= Hexametaphosphate, HA: Hydrofluoric acid

2.10. Identified knowledge gaps

Fe oxides can be a useful tool to increase soil aggregation and tackle the atmospheric increase of C. Most studies have focused on the effect of Fe oxide on stabilising SOC in tropical soils because of their higher Fe oxide quantities compared to that of temperate soils. Previous researches were mostly carried out based on the existing crystallinity of Fe oxide in soil. The capacity of Fe oxide on adsorbing dissolved organic carbon has mostly been studied in forest soils under low or acidic pH. Research suggests that soil organic matter is a dominant aggregating agent in the temperate soils whereas Fe oxide is the main aggregating agent in the tropical soils.

Based on the reviewed literature we have identified the following knowledge gaps.

- The capacity of Fe oxide on organic carbon adsorption under varying land use, texture, organic matter content, and soil pH has not been studied.
- It is little known that how Fe oxide affects soil aggregate formation and SOC sequestration in temperate agricultural soil where the amount of organic matter is relatively lower than other soils.
- The combined effect of organic matter and Fe oxide on soil aggregate formation and SOC stabilisation in temperate soils has not been found.
- The majority of the studies were based on the naturally occurring crystalline condition of Fe oxide in tropical or subtropical soils. How amorphous Fe oxide amendment affects soil aggregation or SOC storage has not been studied.

2.11. Identified experiments on the basis of knowledge gaps

On the basis of knowledge gaps mentioned in Section 2.10., we decided to carry out the following experiments.

1. An initial adsorption study was carried out in the laboratory to see whether Fe oxide can reduce the release of dissolved organic carbon from soil or not (Chapter 4). Soils under varying physical, chemical properties, and land uses were used in this experiment in order to predict the behaviour of Fe oxides as an adsorbent of dissolved organic carbon in different soils.

2. Followed by the short-term initial experiment’s result, a pot experiment was carried out in the laboratory where wheat plants were grown for 8 weeks with an aim to investigate whether Fe oxide amendment can increase the organic carbon storage or not (Chapter 5). Temperate agricultural soils were chosen for this experiment. Furthermore, we incorporated OM in our experiment to investigate the interactive effects of Fe oxide + OM in stabilising SOC. We observed the effect of Fe oxide, OM, and the combined effect of Fe oxide + OM in increasing soil aggregates and organic carbon in each aggregate fraction. SOC was fractionated according to the method suggested by Zimmermann et al. (2007) with minimum alteration as most of the other methods we discussed in our literature could not clarify the functional pool of SOC.

2.12. References

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Chapter 3

Methodologies

3.1. Introduction

All the methodologies used to carry out the experiments in this thesis are described in this chapter.

3.2. Sample collections (soils, ochres, and sewage sludge)

Soils with a different texture, organic matter, pH, and land management practices were collected from the top 20cm from 6 different places. Soils from the Newcastle area werecollected with a particular interest in the availability of kaolinite mineral (according to previous studies by Saidy et al. (2012), the interaction of kaolinite minerals with non-crystalline Fe oxide causes a greater reduction in the amount of SOC mineralisation than that in other soil minerals). Each soil was air-dried, ground, and homogenised by passing through a 2 mm sieve. Sampling location, basic soil properties and land management practices are described in Table 3-1. Soil mineralogical analysis was not carried out with the scope of this project.

Table 3- 1: Sampling Location, land management practice, physical and chemical properties of soils used for the different chapters in this thesis (mean ± standard deviation, n=3).

Field name	**Sampling location**	**Land use**	**pH**	**Organic matter %**	**Texture**	**Chapter**
Nafferton farm	University of Newcastle, Nafferton ecological farm (54° 59’ 12.7” N 1° 53’ 25.4” W)	Arable	6.24 ± 0.04	8.02 ± 0.14	Sandy clay loam	Chapter 5
Big substation east	Leeds University experimental farm, Northern England, (53° 52' 25.2" N, 1° 19' 47.0" W)	Arable-2	7.63 ± 0.29	6.08 ± 0.04	Silt loam	Chapter 4
Siward way	University of York west campus, (53°56'59.4"N 1°03'17.2"W)	Woodland	6.95 ± 0.06	11.33 ± 0.19	Clay loam	
Heslington east	University of York east campus (grid reference SE 63900 50300).	Arable-1	7.80 ± 0.09	5.15 ± 0.16	Silty clay	
Dalham Farm	University of York west campus (53°56'39.0"N 1°03'08.0"W)	Flowerbed	6.98 ± 0.09	5.45 ± 0.24	Loamy sand	
Valley	Leeds University experimental farm, Northern England, (53° 52' 25.2" N, 1° 19' 47.0" W)	Pasture	7.35 ± 0.11	7.99 ± 0.05	Sandy loam	

For the experiments in both Chapter 4 &5, we used ochres as a source of poorly crystalline Fe oxides. Ochres were collected from the drainage site of an abandoned mining site in the UK and supplied to Mark Hodson by the 'UK Coal Authority'. For the experiment in Chapter 5, sewage sludge was used as a source of OM. Sewage sludge (anaerobically digested sludge material which is typically applied to the agricultural land as an organic fertiliser) was available in the laboratory, collected by previous research students from the 'Esholt Water Treatment Plant', Bradford, England.

Ochres and sewage sludge are planned to be characterised at the end of the experiment. However, due to the current Covid-19 situation with closed laboratory the characterisations of ochres and sewage sludge were not possible.

3.3. Soil pH measurement

pH meter was calibrated at pH 4, 7, and 10 by placing the electrode in the buffer solution of pH 4, 7 and 10. The electrode was rinsed with deionised water before each measurement. A mixture of soil and water was prepared at 1:2.5 (soil: water) ratio and shaken for 15 minutes. Soil pH was determined from the mixture (Ministry of Agriculture, Fisheries and Food, 1986) by using an Orion 420Aplus pH meter (Thermo Orion, USA)

3.3. Soil texture determination

Soil texture was determined by following the hand texturing method (Thien, 1979) summarised in Figure 3-1.

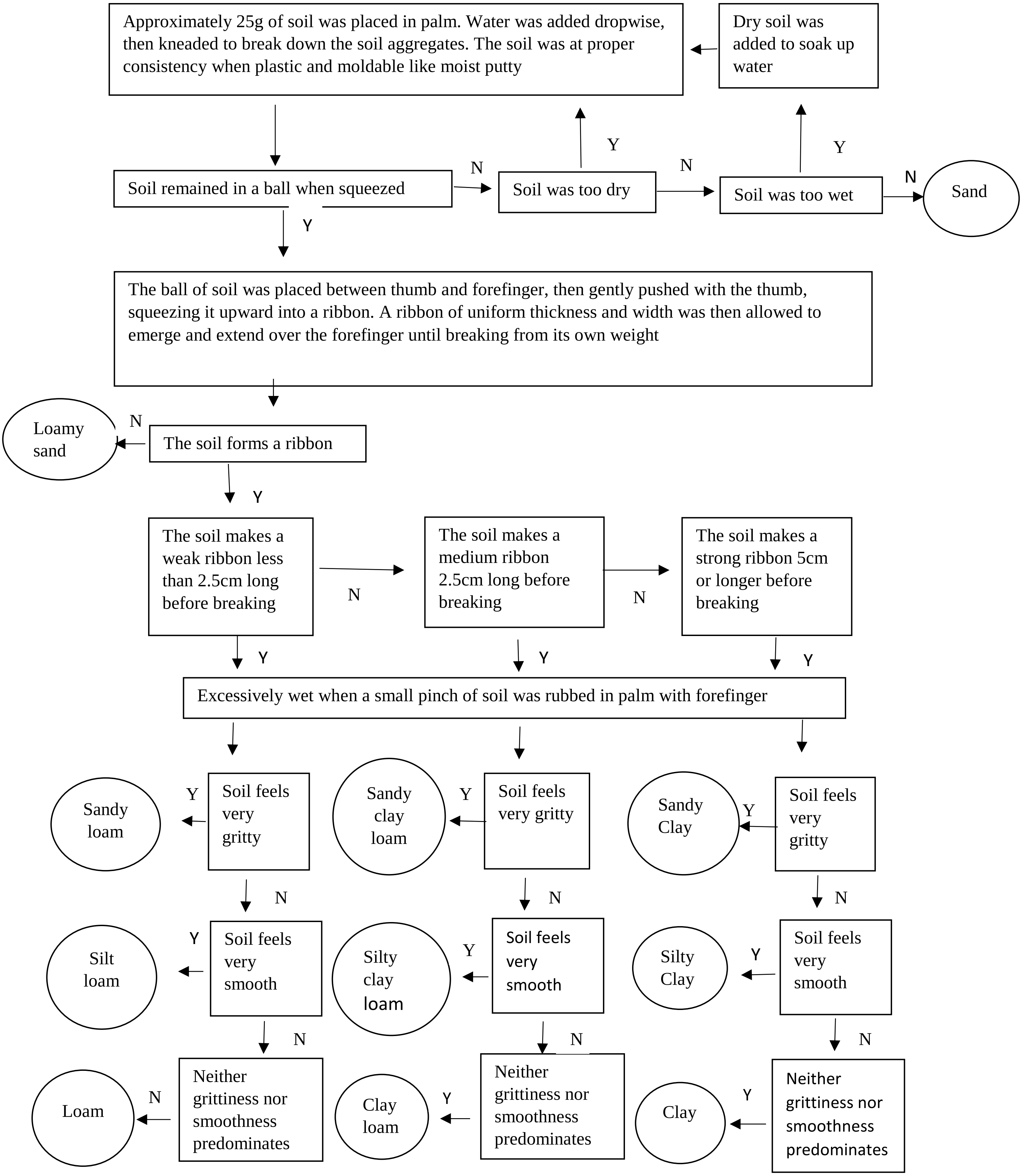

Figure 3- 1: Schematic diagram of soil texture determination by hand feel method (Y= yes, N=no) (Thien, 1979).

3.4. Measurement of soil organic matter

Soil samples were dried at 105° C and the organic matter content was determined by following the loss on ignition method at 550° C (Heiri et al., 2001).

Calculation: Organic matter (%) =

$$\frac{(\text{Dry weight of soil at } 105°\text{C} - \text{Dry weight of soil at } 550°\text{C}) \times 100}{\text{The dry weight of soil at } 105°\text{C}} \qquad \ldots\ldots\ldots\ldots (3.1)$$

3.5. Dissolved organic carbon analysis

8 g air-dried soil sample was accurately weighted to four decimal places. The weighed soil sample was suspended with 40 ml of 0.1 M $CaCl_2$ (1: 5 ratio soil: $CaCl_2$) into a 50 ml centrifuge tube, and shaken for 24 hours by using a multifunctional orbital shaker. The soil suspension was centrifuged at 1000 g for 20 minutes. Then the aliquot was collected and filtered through a 0.45 µm nylon syringe filter. Dissolved organic carbon was determined by using Vario TOC analyser by thermal oxidation with Vario TOC analyser (Dimatoc 2000, Dimatec, Essen Germany).

3.6. Soil fractionation

Soil samples were fractionated by following both physical and chemical fractionation methods modified from Zimmerman et al., 2007 (Figure 3-2.). 30 g of soil was weighed accurately to four decimal places. 150 ml ultrapure water and the weighed soil sample were added in a 250 ml beaker. The soil-water mixture was placed in an ultrasonic disaggregator (Bandelin, Berlin, Germany) to disperse with 22 Jml^{-1} for 3 minutes. Soil particles >250 µm and 63-250 µm were separated by wet sieving. This dispersed soil suspension (>250 µm aperture sieve was placed on top of the 63 µm sieve) was washed with deionised water until the rinsing water became clear. Soil fractions >250 µm and 63-250 µm (sand, stable aggregates and particulate organic matter) were then air-dried first at 40° C and then the oven-dry (at 105° C) weight was recorded. Each of these >250 µm and 63-250 µm dry fractions were mixed properly with approximately 12 ml of Easifloat (a high-density liquid, sodium polytungstate) at a density 1.8 cm^{-3} and centrifuged at 1000 g for 15 minutes. After centrifugation, the light fractions floated on the surface of Easifloat and were collected in pre-weighed metal trays. The heavy fractions at the bottom of the centrifuge tubes were also transferred into pre-weighed metal trays. During the collection of heavy and light fractions, both were intensively washed with deionised water by using a 25µm nylon mesh sieve, in order to make sure that there was no effect of the Easifloat on the mass of these fractions. Oven dry (at 105 °C) weights of both heavy and light fractions were taken. The light fraction has been described as particulate organic matter and the heavy fraction as sand and stable aggregates (S+A) (Zimmerman et al., 2007).

All the water used from wet sieving was collected and centrifuged at 3000 g for 10 minutes by using 250 ml centrifuge tubes. After centrifugation, the volume of all the water used was recorded by using a 4 L measuring cylinder and a subsample was filtered through a 0.45 µm nylon syringe filter. The amount of dissolved organic carbon in the aliquot of the filtrate was determined by thermal oxidation with Vario TOC analyser (Dimatoc 2000, Dimatec, Essen, Germany). The solid fractions from all the centrifuge tubes (0.45 µm- 63 µm) were washed and transferred into a pre-weighed single centrifuged tube and then re-centrifuged. Then the aliquot was drained off. The total weight of the centrifuge tube with the solid sediment fraction was recorded. The weight of the wet sediment was determined by deducting the weight of the centrifuge tube from the total weight. A subsample of the sediment (0.45 µm- 63 µm solid fraction) was placed into a pre-weighed metal tray, oven-dried at 105 °c and weighed. The total dry mass of 0.45 µm- 63 µm solid fractions were recorded by calculating the amount of moisture loss from the subsample and adjusting that value with the total initial mass of the wet sediment. In order to separate a chemically resistant carbon fraction, 1 g of soil was taken from the dry 0.45 µm- 63 µm fraction and oxidised with 50 ml of 6% NaOCl (adjusted to pH 8 with concentrated HCl) for 18 hours at room temperature. Then the oxidation residue was centrifuged at 1000g for 15 minutes, decanted, washed with deionised water and centrifuged again. This oxidation step was repeated twice. Carbon content in the solid fractions was determined by combustion with an elemental analyser (Vario EL Elementar Hanau, Germany).

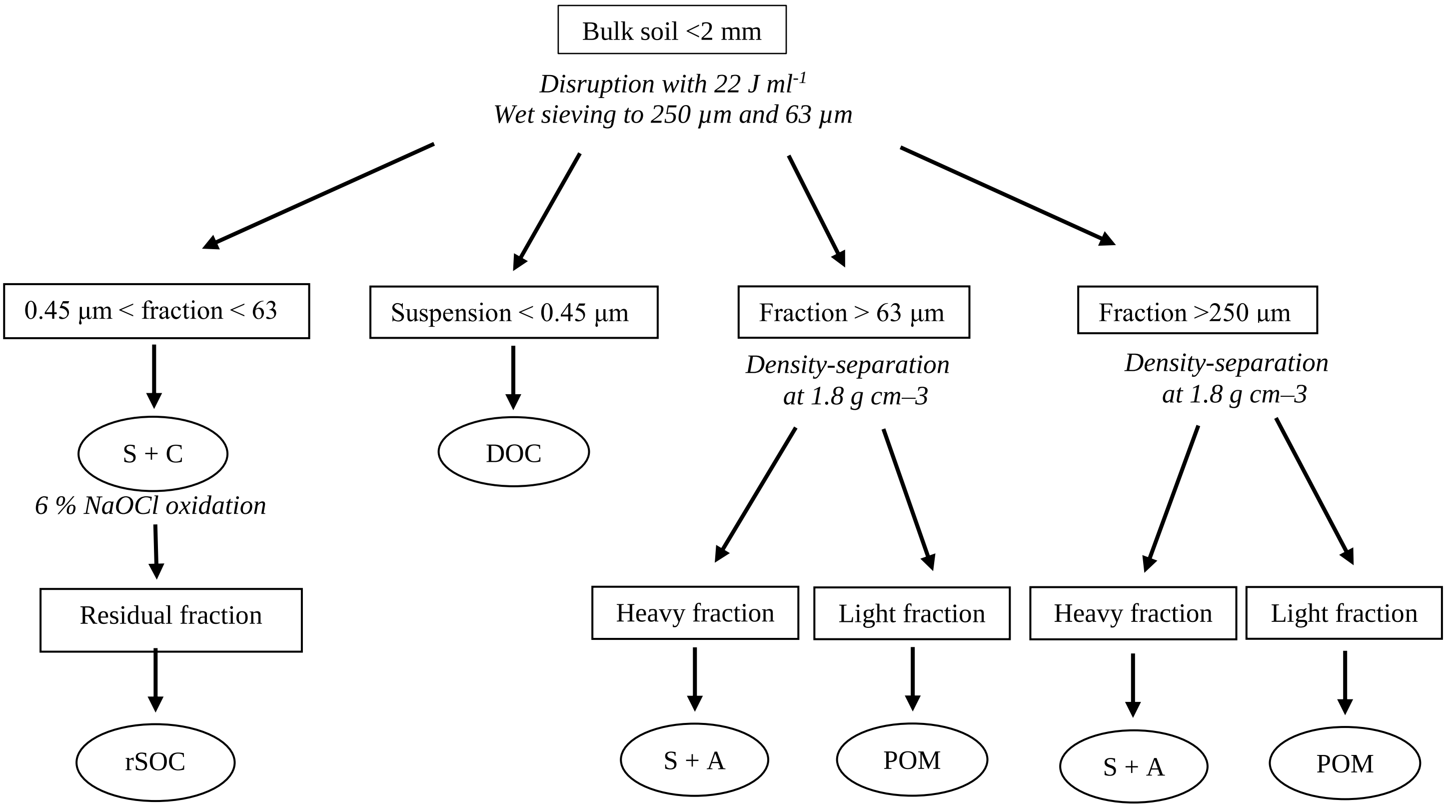

Figure 3- 2: Diagram of the fractionation procedure; S + C = silt and clay, rSOC = resistant soil organic carbon, DOC = dissolved organic carbon, S + A = sand and stable aggregates, and POM = particulate organic matter (a modified version of fractionation procedure of Zimmerman et al., 2007; modified by Hodson E.M., 2019).

3.7. Soil respiration measurement

Soil respiration is defined as the consumption of oxygen by soil organisms during chemical oxidation of carbon compounds in order to generate energy (Lloyd and Taylor, 1994). Soil respiration rate can be measured by the amount of oxygen decline (%) per minute in soil. Optical fluorescence-based sensors commercially known as Loligo sensors were used to measure dissolved O_2 concentrations in the soil slurry. After harvesting the wheat plants (wheat plants were grown for 8 weeks for the experiment in Chapter 5) fresh soil samples were taken for the respiration measurement to reduce any unwanted changes in microbial activity. Soil and deionised water were kept inside an incubator at 20° C for 3 hours before the measurement (in order to minimise the temperature effect). Soil slurries were prepared by mixing the soil and deionised water at a 1:2.5 (mass of soil: volume of water) ratio. A wide-bore pipette tip was used to pipette 600µl of soil slurry to fill each well in the loligo plate. Loligo plates with completely filled wells were kept inside the incubator. The SDR (Sensor Dish Reader-v38) software was connected to the plate reader which was already connected to the laptop from the incubator. Measurement of O_2 consumption rate in each soil sample is summarised in Figure 3-3. The measurement interval was 3 minutes. Loligo output in the excel sheet showed the rate of O_2 consumption in each well. As the starting values of the O_2 consumption rate were varied with initial microbial activity, results for the first few minutes were ignored. A linear phase of decline in O_2 concentration was selected for each well. ($r^2= 0.99$, $P\leq0.05$). The slope of each well was used to calculate the O_2 consumption rate (Yashchenko et al., 2016)

Calculation: microbial activity, % O_2 consumption $s^{-1}= \{(\text{slope}_{\text{sample}} – \text{slope}_{\text{blank}}) \times -1\}$… (3.2)

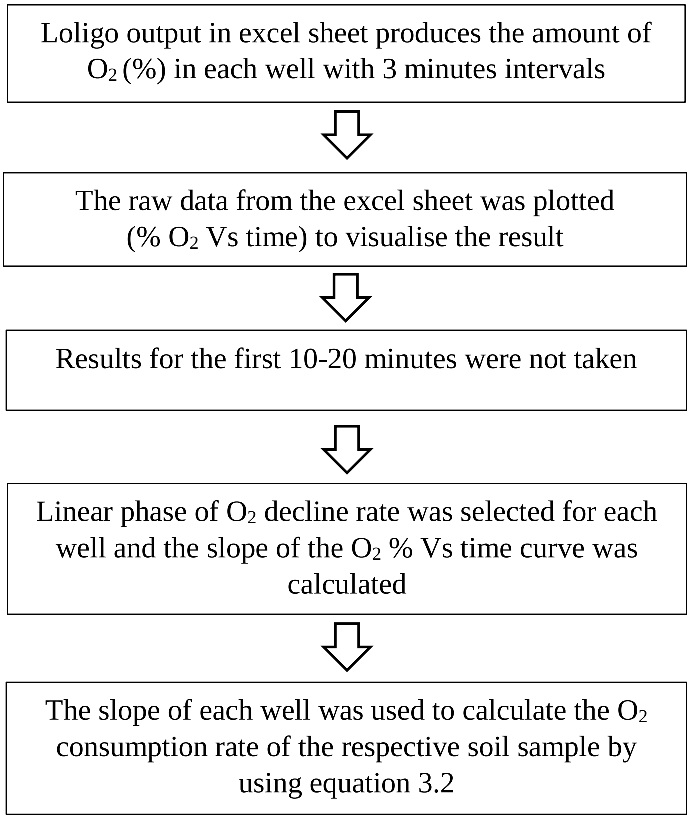

Figure 3- 3: Flowchart of the measurement of O_2 (%) consumption rate in soil.

3.8. Measurement of dithionate extractable Fe

The dithionite-citrate method was used to determine the concentration of extractable Fe in soils (Canadian Agricultural Services Coordinating Committee, 1998). 0.5 g of <2mm air-dried soil was weighed, passed through 0.15 mm (100mesh) sieve and put into a 50 ml centrifuge tube. 25 ml of 0.68 M sodium citrate ($Na_3C_6H_5O_7 . 2H_2O$) solution and 0.4g of sodium dithionite ($Na_2S_2O_4$) were added. The soil suspension was shaken for 16 h in an end-over-end shaker and then centrifuged for 20 minutes at 510 g. The aliquot was filtered and diluted by the factor 1000. Dilution of Fe extracts and the preparation of standard solutions were made by using the extracting solution at the same concentration. Inductively coupled plasma optical emission spectrometer (ICP-OES) was used to determine the amount of available Fe in soil.

Calculation

$$\% \text{ Fe} = \frac{\mu g/ml\ in\ final\ solution \times extractant(ml) \times dilution}{sample\ weight(g) \times 10{,}000} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (3.3)$$

3.9. Quality control and Statistical analysis

A certified reference solution (in house 500 ppb solution) was analysed to determine the accuracy of Fe analysis. The accuracy was 102-104%. The detection limit for the analysis of extractable Fe was 0.006894 mg/L calculated from the mean plus 6 times the standard deviation of the ten replicate analyses of the blank calibration standard (Gill, 2014). Analytical precision was 0.4 calculated from the duplicate analysis of 10% of the samples divided by (Gill, 2014) by using equation 3.4.

$$\text{Analytical precision} = \frac{(\text{Difference between the concentration in 1st analysis and 2nd analysis}) \times 100}{\text{mean values of 1st analysis and 2nd analysis}}$$

$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (3.4)$$

For the accuracy of dissolved organic carbon analysis a certified reference solution (50 mg/L organic carbon solution) was analysed by Vario TOC analyser (Dimatoc 2000, Dimatec, Essen, Germany) as a calibration standard. The accuracy was 104.8-105.4%. Detection limit and analytical precision were .880692 mg/L and 0.51 respectively (calculation stated above).

For the accuracy of total organic carbon analysis, a certified reference material [a high organic content sediment (48.09% organic carbon) called Brich leaf] was analysed by combustion with an elemental analyser (Vario EL Elementar Hanau, Germany) as a calibration standard. The accuracy was 103.5%. Detection limit and analytical precision were 0.2003234 (%) and 0.45 respectively (calculation stated above).

All the statistical tests carried out in this thesis are summarised in Table 3-2. Holm Sidak method was used for all pairwise multiple comparisons followed by the ANOVA tests. Normality and the equal variance of the data were tested by Shapiro-Wilk method and Brown-Forsythe method respectively. P values of ≤0.05 were used as the critical threshold of significance for all the tests. All these computations were made by using Sigma Plot standard package, (released 2018, version 14.0). Graphs were produced by using Microsoft excel.

Table 3- 2: Summary of all the statistical tests carried out for this thesis.

Chapter	Statistical test	Factors for AONVA test	Variable for ANOVA test
Chapter 4	Two-way analysis of Variance (ANOVA)	(1) Different level of Fe oxide (2) Different soil type	Concentration of DOC reduction (%)
	Linear Regression analysis for adsorption isotherm plots		
Chapter 5	Two-way analysis of variance	(1) Different level of Fe oxide (2) Different level of organic matter amendment	Soil respiration rate
	Two-way analysis of variance	Same above used for soil respiration rate	Dry matter biomass of wheat plants
	Three-way analysis of variance	(1) Different level of Fe oxide (2) Different level of organic matter amendment (3) Experimental time	Soil pH
	Three-way analysis of Variance	Same above used for soil pH	Extractable Fe oxide
	Three-way analysis of variance	Same above used for soil pH	Mass of soil aggregate fractions
	Three-way analysis of variance	Same above used for soil pH	Concentration of organic carbon (mg/g soil) in soil fraction
	Three-way analysis of variance	Same above used for soil pH	Mass of total organic carbon (mg) in soil fraction
	Spearman’s rank correlation between interrelated soil properties		

3.10. References

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Chapter 4

Can Fe oxide reduce the release of organic carbon from soil?

4.1. Introduction

Dissolved organic carbon (DOC) in soil originates from recent plant litter, microbial biomass, root exudates or humic substances (Kalbitz et al., 1999) and is considered to be the most available form of organic carbon for biological degradation (Boddy et al., 2007). Adsorption of dissolved organic carbon (DOC) by poorly crystalline Fe oxide is an important mechanism for SOC storage and stabilisation (Oades, 1998; Mikutta et al., 2014). Mechanism of DOC adsorption on Fe oxide surface is highly heterogeneous and largely dependent on the nature of DOC, soil solution pH, temperature, ionic strength, and the available reactive surface of Fe oxide (McLean and Bledsoe 1992; Oades, 1988). Several studies suggest that ligand exchange between carboxyl or hydroxyl group of DOC and Fe oxide surface is a dominant mechanism of DOC adsorption, particularly under acidic pH (Murphy et al., 1990; Parffitt et al., 1997; Davis et al., 1982). Research suggests that 72-92% of adsorbed DOC are irreversibly bound to Fe oxides (Gu et al., 1994). Previous studies were mostly carried out in temperate forest ecosystems under acidic pH (Porras et al., 2017; Guggenberger et al., 1993; Solinger et al., 2001; Currie et al., 1996). Little is known about the adsorption of DOC by Fe oxide under varying land use, soil pH and texture. In this chapter, we aimed to investigate the adsorption of DOC by poorly crystalline Fe oxides in variety of soils.

The main hypothesis was

- Fe oxide amendment would adsorb DOC in soil and reduce the release of DOC across all soil types.

4.2. Method

4.2.1. Measurement of % reduction of DOC

The ability of Fe oxides to adsorb dissolved organic carbon was determined by the measurement of DOC followed by the calculation of the percentage reduction of DOC (shown in equation 4.1.) relative to the control soil. Soil samples from five different places (Heslington East, Dalham Farm, Big substation east, Valley farm, and Siward Way) were used for this measurement. Based on the type of land use we named soils from Heslington East= Arable-1, Dalham Farm=Flowerbed, Big substation east= Arable-2, Valley farm =Pasture, and Siward Way= Woodland soil. Moist ochre (as a source of Fe oxides) was oven-dried at 105° c and ground with pestle and mortar. Soils were amended with different level of Fe oxides (0%, 0.5%, 1%, 2% and 4%). The detailed procedure of soil sample collection and the basic properties of those soils are described in Chapter 3 (Section 3.2.).

Reduction of DOC (%) =

$$\frac{\text{Mean concentration of DOC in unamended soil } (mg/g\ soil) - \text{ the concentration of DOC in Fe oxide amended soil } (mg/g\ soil)}{Mean\ concentration\ of\ DOC\ in\ unamended\ soil\ (mg/g\ soil)} \times 100 \ldots\ldots (4.1)$$

4.2.2. Measurement of DOC adsorption by Fe oxide

The amount of DOC adsorbed by Fe oxide was determined by the following equation (Spark, 2003)

$$q = \frac{(C_0 - C_f) \times V}{m} \ldots\ldots (4.2)$$

q= amount of dissolved organic carbon adsorbed per unit mass of Fe oxide (mg/kg)

C_0= the initial concentration of DOC mg/L

C_f = final concentration of DOC mg/L

V= adsorptive volume (L)

m= mass of Fe oxide (kg)

In order to describe the relationship between the equilibrium concentration of DOC and the amount of DOC adsorbed by Fe oxide at a constant temperature, we used three different types of adsorption isotherm.

Linear adsorption isotherm

Linear adsorption isotherm describes that adsorbate molecules or ions are distributed or partitioned at the solid adsorbent surface from the bulk solution (the solution that contains the adsorbate molecules) without any specific bonding mechanism (Chiou et al., 1977). It assumes that there is no competition between multiple adsorbates for the adsorption sites. It describes a reversible adsorption process using the following equation

$$Cs = K_d Ceq \text{……………………………………………………………………………} (4.3)$$

Where,

Cs= amount of material adsorbed per unit mass of adsorbent mg kg^{-1}

K_d= partition coefficient Lkg^{-1}

Ceq= equilibrium concentration of adsorbate mg L^{-1}

Langmuir adsorption isotherm

Langmuir adsorption isotherm describes that when the concentration of adsorbate increases, the available adsorption site of the adsorbent decreases and adsorption reaches to a maximum when all the adsorption sites are saturated by the adsorbate (Spark, 2003). It assumes that adsorption takes place on a homogenous surface where a single molecule of adsorbate interacts with a single site of the adsorbent (Liu et al., 2019). It can be described by the following equation

$$\frac{Cs}{Ceq} = \frac{bCsm}{1 + Ceqb} \text{………………………………………………………………..…} (4.4)$$

Cs = quantity of adsorbate adsorbed by the solid (mg kg^{-1})

Ceq = equilibrium solution concentration (mg L^{-1})

b = binding constant (L mg^{-1}), depends on the chemical nature of the adsorbent and describes the free energy of adsorption.

Csm = maximum quantity adsorbable (mg kg^{-1})

1/Cs was plotted against 1/Ceq for each soil type. Csm and b were calculated from the slope (1/bCsm) and the intercept (1/Csm) of the isotherm.

Freundlich adsorption isotherm

Freundlich adsorption isotherm cannot measure maximum adsorption like Langmuir adsorption (Sparks, 2003). It describes that at low adsorbate concentration the amount of adsorbate adsorbed per unit mass of adsorbent increases with the increase in adsorbent concentration whereas at high adsorbate concentration adsorption reaches to a constant value (Proctor and Vazquez, 2009). Freundlich adsorption isotherm assumes that adsorption takes place on a heterogeneous surface. The linearised Freundlich adsorption equation can show multiple slopes which explains the possibility of having different binding sites where adsorption takes place with varying energy for each site (Sparks, 2003). It is expressed in the following equation.

$\log Cs = 1/n \log Ceq + \log K_d$ ……………………………………………………………….. (4.5)

This equation follows the equation for a straight line when LogCs was plotted against LogCeq

Where,

Cs = quantity adsorbed by the adsorbent (mg kg^{-1})

Ceq = equilibrium solution concentration (mg L^{-1})

n= constant and 1/n = slope of the straight line

K_d = distribution coefficient= intercept of the straight line.

4.3. Results

4.3.1 Reduction of DOC release in different soil types

The concentrations of DOC at different Fe oxide treatments within different soil types are presented in Figure 4-1. The two-way analysis of variance test showed that DOC concentration reduced significantly ($P \leq 0.05$) between different soil types and within different Fe oxide treatments. There was a significant interaction ($P \leq 0.05$) between Fe oxide levels and soil types (Table 4-1). Relative reduction of DOC concentration (%) due to the interaction of Fe oxide and soil types are shown in Table 4-2.

Table 4- 1: Two-way analysis of variance results of the effects of Fe oxide and soil types on % reduction of DOC concentration. Data passed the normality test (P= 0.613) and equal variance test (P=0.287).

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Soil type	4	0.0160	0.00399	407.898	<0.001
Fe oxide	3	0.00474	0.00158	161.470	<0.001
Soil type * Fe oxide	12	0.00303	0.000252	25.769	<0.001

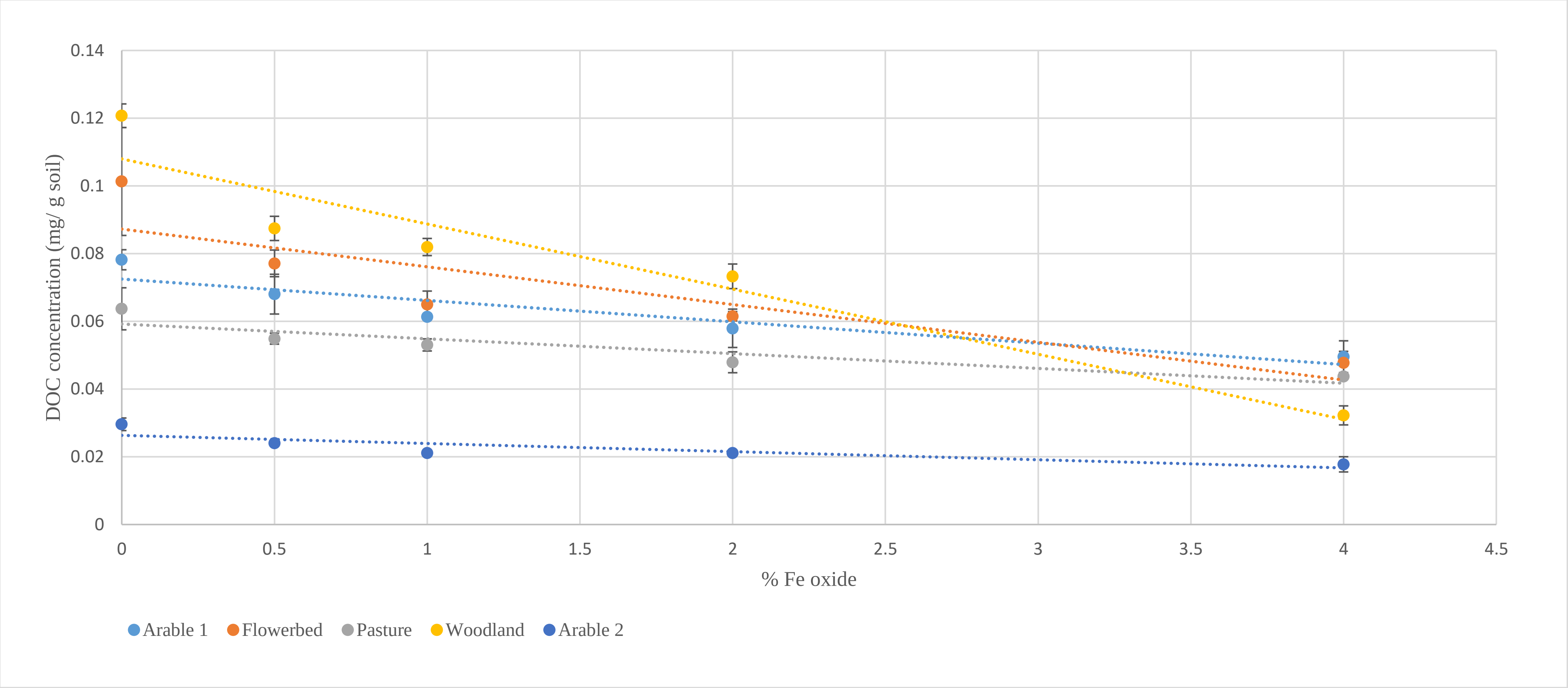

Figure 4- 1: Concentration of DOC (expressed in mg of C per g mass of soil) at various Fe oxide level within Arable-1, Flowerbed, Arable-2, Pasture and Woodland soil types (n=3, error bar= ± Standard deviation). The dotted lines represent the linear regression of the data for Arable -1 (R^2=0.8568, P<0.01), Flowerbed (R^2=0.7655, P<0.01), Arable-2 (R^2=0.7328, P<0.01), Pasture (R^2=0.8371, P<0.01), and Woodland soil (R^2=0.915, P<0.01).

Table 4- 2: Reduction of DOC (%) relative to the DOC concentration at control treatment (no Fe oxide amendment) in different soil types at different Fe oxide level. Values are means of 3 replicates ± standard deviation. Lowercase letters are for the comparisons within Fe oxide treatments and uppercase letters are for the comparisons within soil types. Values followed by the same letter are not significantly different (P>0.05)

Fe oxide (%)	Arable-1	Arable-2	Pasture	Flowerbed	Woodland
0.5	12.98±7.51aEC	18.71± 2.83aDEB	13.84±2.54aCD	23.87±3.88aB	27.55±2.95aADB
1	21.53±1.13bcED	28.63±0.46bcDBA	16.74±2.80abcCE	35.87±3.92bcB	32.10±2.09abcAB
2	25.88±7.24cED	28.82±0.10cDC	24.80±4.83cdCE	39.27±1.34cB	39.27±3.01cAB
4	36.61±5.99dECD	39.89±7.63dD	31.35±1.02dC	52.85±3.34dB	73.29±2.32dA

Pairwise multiple comparisons found that the concentration of dissolved organic carbon significantly reduced with each Fe oxide treatment in all the soil types (P<0.001). Woodland soil (contained the highest amount of organic matter) had the greatest decrease in DOC concentration due to Fe oxide treatment compared to any other soil types. On the other hand, the Pasture soil and Arable-1 soil type had the lowest reduction in DOC concentration. Arable -1 soil had the lowest amount of organic matter and Pasture soil had the second-highest amount of organic matter among all the soil types. This result indicates that the amount of OM in soil did not consistently affect the adsorption of DOC by Fe oxides. The significant decrease in DOC concentration in each soil type can be expressed with this increasing order: Arable-1 & Pasture <Arable-2<Flowerbed<Woodland (P<0.05).

For each Fe oxide treatment, the reduction in DOC was significantly greater in the Woodland soil and Flowerbed soils than in Arable-1 and Pasture soils. Higher levels of Fe oxide treatment (2% and 4%) showed a significantly greater reduction in DOC in the Woodland and Flowerbed than that in Arable-2 soil. The reduction of DOC was not significant between Arable-2 and Pasture soil at low level of Fe oxide. 4% Fe oxide treatment caused greater (%) reduction of DOC in Arable-2 soil than in Pasture soil. Woodland soil had the greatest decrease in (%) reduction of DOC among all other soil types.

4.3.2. Adsorption of DOC by Fe oxide

Three different adsorption isotherms were used (Section 4.2.2.) in order to see which one can better describe the DOC adsorption by Fe oxide.

Linear adsorption isotherm

Linear adsorption isotherm of DOC on Fe oxide surfaces for five different soil types is shown in Figure 4-2. Linear adsorption parameters and regression analysis summary for each soil type are presented in Table 4-3.

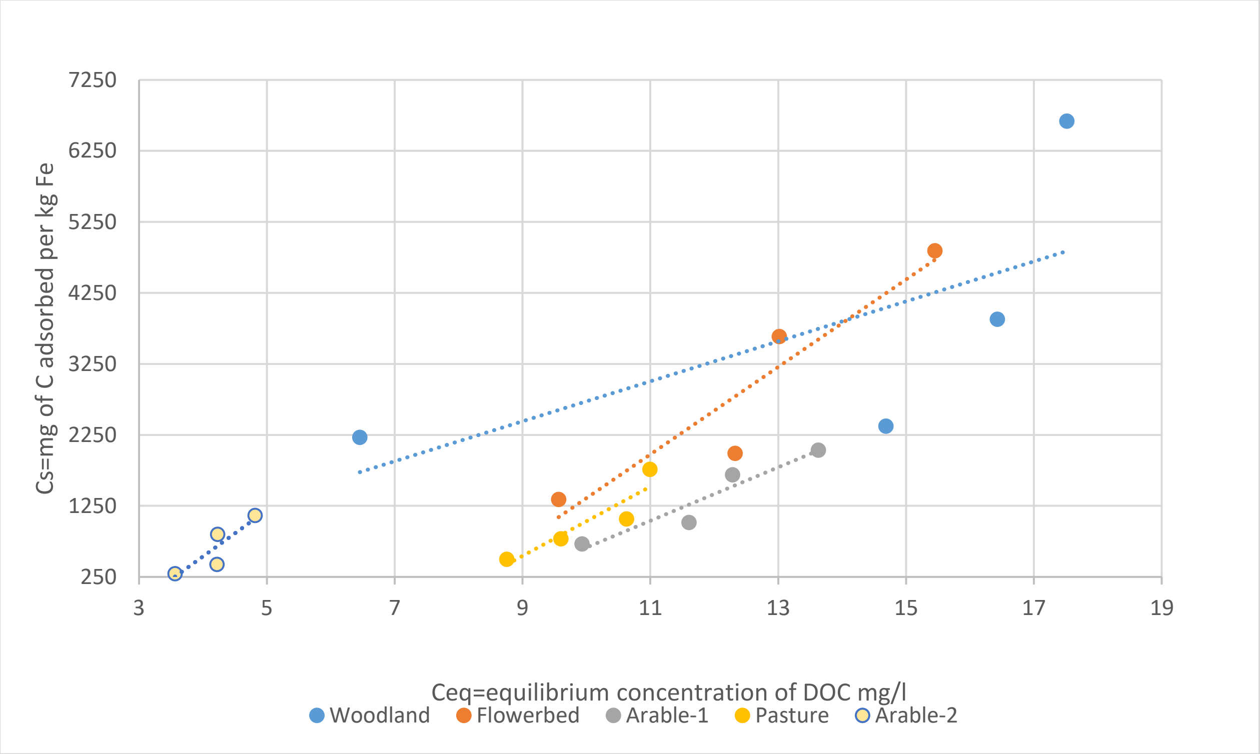

Figure 4- 2: Linear adsorption isotherm of DOC on Fe oxide surface for Arable-1, Flowerbed, Arable-2, Pasture, and Woodland soil. The dotted lines represent the linear regression of the data for Arable -1 (R^2=0.9121, P<0.045), Flowerbed (R^2=0.8822, P<0.061), Arable-2 (R^2=0.7819, P<0.116), Pasture (R^2=0.8355, P<0.086), and Woodland soil (R^2=0.4676, P<0.316).

Table 4- 3: Linear adsorption parameters used in the equation 4.3 and the summary of regression analysis for the adsorption isotherm plot in Figure 4-2 (uncertainty values indicate standard error).

Soil type	Slope = Kd (Partition co-efficient)	P
Arable-1	376±82.	0.045
Flowerbed	615±159.	0.061
Arable-2	651±243.	0.116
Pasture	488±153.	0.086
Woodland	281±212.	0.316

Woodland soil showed the lowest Kd value and Arable-2 soil had the highest Kd value (Table 4-3). There was no significant correlation between the amount of C adsorbed (mg/kg Fe oxide) and the equilibrium concentration of DOC (mg/L) in all the soil types except Arable-1 (Figure 4-2, Table 4-3).

Langmuir adsorption isotherm

Langmuir adsorption isotherm for the adsorption of DOC on Fe oxide surface for five different soil types is shown in Figure 4-3. Langmuir adsorption model parameters and regression analysis summary for each soil type are presented in Table 4-4.

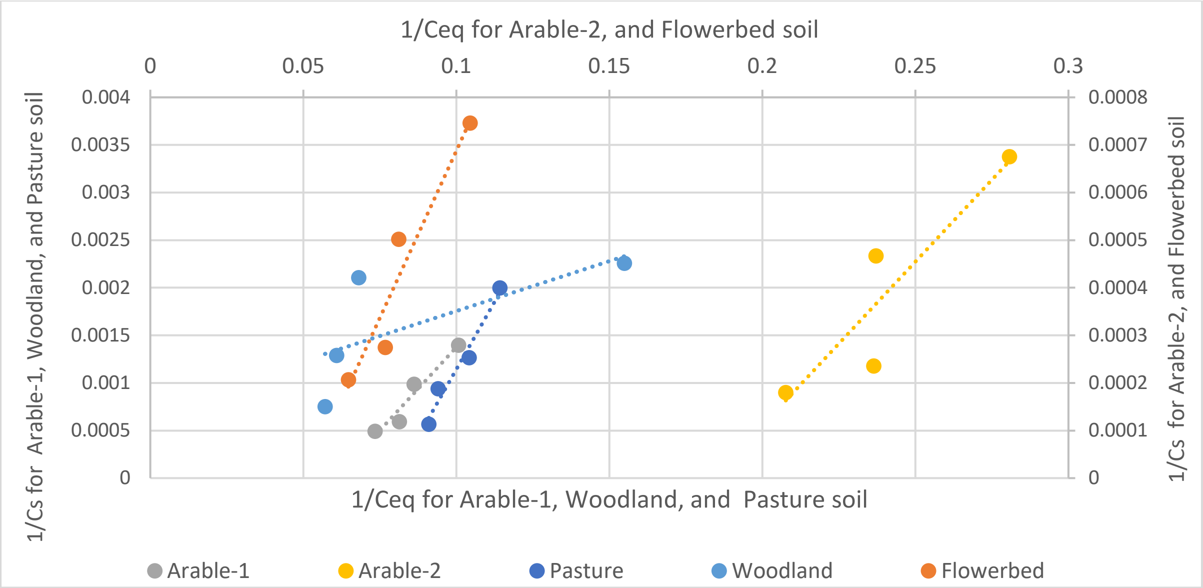

Figure 4- 3: Langmuir adsorption isotherm of DOC on Fe oxide surface for Arable-1, Flowerbed, Arable-2, Pasture, and Woodland soil. The dotted lines represent the linear regression of the data for Arable -1 (R^2=0.9446, P<0.028), Flowerbed (R^2=0.9213, P<0.040), Arable-2 (R^2=0.8306, P<0.089), Pasture (R^2=0.9674, P<0.016), and Woodland soil (R^2=0.4774, P<0.309).

Table 4- 4: Langmuir adsorption parameters used in the equation 4.4 and the summary of regression analysis for the adsorption isotherm plot (uncertainty values indicate standard error) (Figure 4-3).

Soil type	Intercept = 1/Csm	Slope= 1/bCsm	Maximum amount of DOC adsorbed = Csm (mg kg-1)	Binding constant = b (Lmg^{-1})	P
Arable-1	-0.00212±0.000515	0.0349±0.00598	-471.957	-0.06064	0.028
Flowerbed	-0.00072±0.000241	0.0140±0.00290	-1394.82	-0.05104	0.040
Arable-2	-0.00633±0.00266	0.0344±0.0110	-158.02	-0.18391	0.089
Pasture	-0.00453±0.000747	0.0568±0.00737	-220.567	-0.07986	0.016
Woodland	0.000141±0.000146	0.00210±0.00155	7079.092	0.067347	0.309

Our result showed that DOC adsorption data for all the soil types except Woodland soil has a good geometric fit ($P<0.05$) with Langmuir isotherm curve (Figure 4-3). However, the intercept of the isotherm curve for all the soil types except Woodland soil is negative (Table 4-4). This result did not make any chemical sense as the maximum adsorption cannot be negative (Table 4-4).

Freundlich adsorption isotherm

Freundlich adsorption isotherm for DOC adsorption on Fe oxide surfaces for five different soil types is shown in Figure 4-4. Freundlich adsorption model parameters and regression analysis summary for each soil type are presented in Table 4-5.

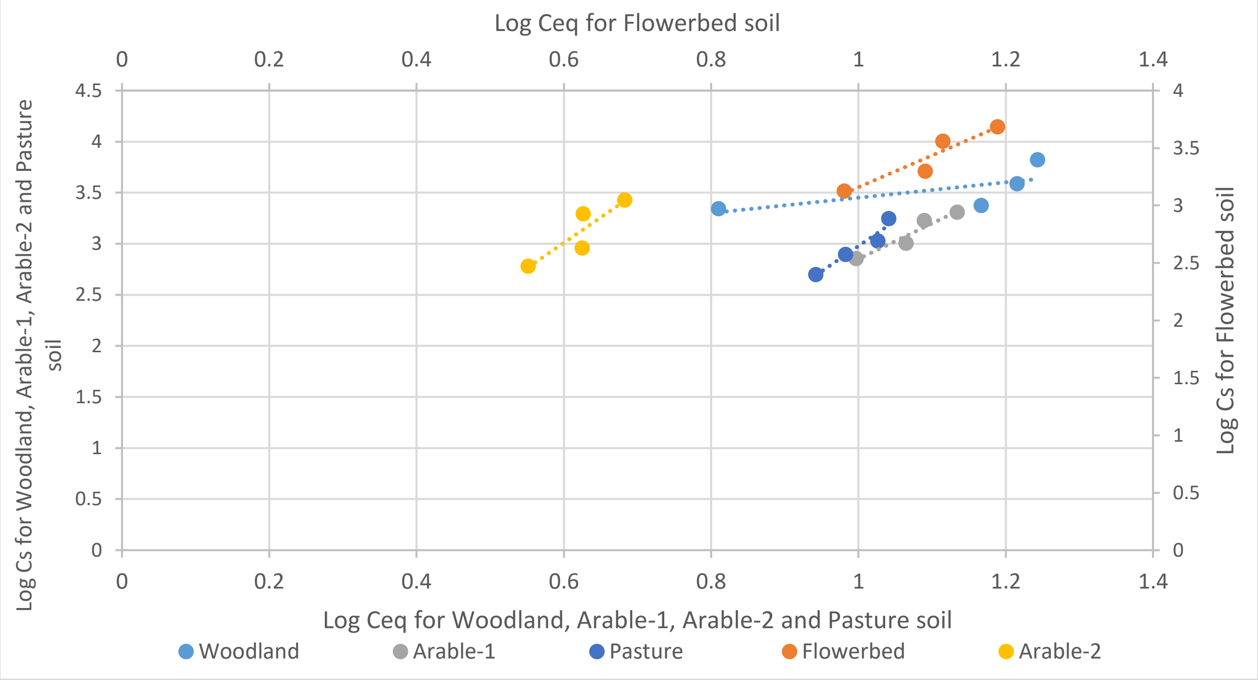

Figure 4- 4: Freundlich adsorption isotherm of DOC on Fe oxide surface for Arable-1, Flowerbed, Arable-2, Pasture, and Woodland soil. The dotted lines represent the linear regression of the data for Arable -1 ($R^2=0.927$, $P<0.037$), Flowerbed ($R^2=0.8952$, $P<0.054$), Arable-2 ($R^2=0.7962$, $P<0.108$), Pasture ($R^2=0.9304$, $P<0.035$), and Woodland soil ($R^2=0.4685$, $P<0.316$).

Table 4- 5: Freundlich adsorption parameters used in the equation 4.5 and the summary of regression analysis for the adsorption isotherm plot in Figure 4-4 (uncertainty values indicate standard error)

Soil type	Intercept = Log Kd	Slope=1/n	P
Arable-1	-0.6247±0.738	3.476±0.688	0.037
Flowerbed	0.395±0.733	2.763±0.669	0.054
Arable-2	0.036±0.981	4.399±1.574	0.108
Pasture	-1.97±0.956	4.947±0.957	0.035
Woodland	2.699±0.636	0.752±0.567	0.316

Similar results like Langmuir adsorption isotherm was found. We observed the slope of the Freundlich isotherm curve (1/n) is > 1 in all the soil types except Woodland soil (Table 4-5). This indicates a good geometric fit of DOC adsorption data with Freundlich isotherm but no chemical sense because adsorption cannot be infinitive for a minimal increase in Ceq. There was a significant correlation (P=<0.05) between Log Cs and Log Ceq only in Arable-1 and Pasture soil (Table 4-5, Figure 4-4).

4.4. Discussion

In this experiment, the concentration of DOC significantly reduced with an increase in Fe oxide level within all the soil types (Figure 4-1). This result supports the findings of Kalbitz et al. (1999) who found that the adsorption of DOC was quantitatively related to the amount of Fe oxides in soil. The highest amount of DOC reduction was observed for Woodland soil. Woodland soil contained the highest amount of OM compared to other soil types. It released a higher amount of DOC in the control soil (unamended Woodland soil) compared to that in other soil types which had a relatively low amount of OM (Figure 4-1, DOC concentration at 0% Fe oxide treatment). Therefore, the difference in DOC release between the control soil and the Fe oxide treated soil was the greatest for Woodland soil whereas in other soils reduction in DOC was limited as there was less DOC for release into the control soil solution and consequently less adsorption of DOC by Fe oxide at steady state (Tipping, 1998; Qualls et al., 1991). In addition, Woodland soil had a pH value of 6.95. Previous studies by Honghai et al. (2008) found that ligand exchange is the predominant mechanism of DOC adsorption on Fe oxide surface which occurs when the soil pH is below 7.5. Several other studies suggested that ligand exchange between carboxyl or hydroxyl group of DOC and Fe oxide surface occurred at slightly acidic pH in soil (Murphy et al., 1990, Parffitt et al., 1997, Davis et al., 1982). Therefore, the pH level in Woodland soil favoured greater adsorption of DOC compared to that in other soil types which had a relatively higher soil pH value. The fact that acidic pH favours the adsorption of DOC on Fe oxides surfaces can again be used to support the reason behind Flowerbed soil (pH 6.98) having higher reduction of DOC due to Fe oxide treatment than that in Arable-1, Arable-2 and Pasture soil (pH value 7.80, 7.63 and 7.35 respectively). Our result showed that although Pasture soil had a higher amount of OM compared to Arable -2 and Flowerbed soil (Chapter 3, Table 3-1.), the amount of DOC reduction by Fe oxide treatments in Pasture soil was significantly lower compared to Arable-2 and Flowerbed soil. Research suggests that soil type has an important role in the adsorption mechanism. Pasture soil texture was sandy loam, indicating the presence of a high amount of sand particles and a subsequent lack of clay content. Flowerbed soil texture was loamy sand indicating more sand than Pasture soils. Soils containing a high amount of sand particles and a subsequent lack of clay content have less capacity to adsorb organic matter due to the lack of adequate surface area and low CEC (Abdulgawad et al., 2009; Shing and Shing, 2008). Therefore, the release of DOC into the soil solution was relatively the greatest in Flowerbed soil followed by the Pasture soil and the lowest in Arable-2 soil (Arable-2 soil had silt loam texture) among these three soil types (Figure 4-1, the concentration of DOC at 0% Fe oxide treatment). Higher release of DOC into control soil and slightly acidic pH in Flowerbed soil favoured significantly higher percentage reduction of DOC due to Fe oxide amendment compared to that in Pasture soil. At a low level of Fe oxide treatment, there was no significant difference in % reduction of DOC between Pasture soil and Arable-2 soil. However, Arable-2 soil showed a significantly higher percentage of DOC reduction at a high level of Fe oxide treatment (4% Fe oxide) than that in Pasture soil. A possible explanation for this result could be the fact that in Pasture soil there was a presence of high concentration of anions like phosphate or sulphate that competed for the adsorption sites and reduced the capacity of Fe oxides to adsorb DOC (Gu et al., 1994). Furthermore, the composition of the DOC also controls the adsorption reaction. DOC that contains aromatic rings, amino acid, S- or N- containing group is preferentially sorbed by soil minerals (McKnight et., 1992). In Arable soil, organic fertilisation such as animal manure, sewage sludge application has a significant impact on soil microbial community which in turn affect the composition of soil organic matter (Zhu et al., 2016). We assume DOC in Arable-2 might contain a higher amount of aromatic rings, amino acid, S- or N- containing group than that in Pasture soil. Therefore Pasture soil had significantly lower percent reduction of DOC than that in Arable-2 soil even though it contained higher amount of OM.

Results for the fitting of our DOC adsorption data to adsorption isotherm plots showed that only Woodland soil showed a chemically meaningful fit in both Langmuir adsorption isotherm and Freundlich isotherm. We found the maximum adsorption could be 7079.092 mg of C per kg Fe oxide (Table 4-4) from the Langmuir equation. Data for DOC adsorption on Fe oxide surface in Arable-1, Arable-2, Flowerbed and Pasture soil showed a good geometric fit with both Langmuir isotherm and Freundlich isotherm but it could not be justified from a chemical perspective. Data for the adsorption of DOC by Fe oxides fitted well to the linear adsorption isotherm only for Arable-1 soil (R^2= 0.912, P=0.045). Results for the all pairwise multiple comparisons showed that Arable-1 soil had the lowest percentage reduction of DOC within Fe oxide treatments among all other soil types (Section 4.3.1), indicating that Arable-1 soil had least adsorption capacity compared to other soil types. Our result supports the previous finding that DOC adsorption shows the best fit with linear adsorption isotherms only in soils which have little adsorption capacity (little adsorption capacity means either the release of DOC to the soil solution is low or lack of available adsorption sites) (Jeroen et al., 2007). Woodland soil had the lowest partition coefficient (Kd) value whereas Arable-2 soil had the highest Kd value (Table 4-3). This result pointing towards the lower affinity of DOC to Fe oxide surface in Woodland soil and higher affinity of DOC to Fe oxide surface in Arable-2 soil compared to other soil types (Honghai et al., 2008). Previous studies suggested that DOC shows a strong affinity to Fe oxide surface at low organic matter content in soil (Kilbitz et al., 2000). Therefore, Woodland soil which had the highest amount of OM, showed a lower affinity of DOC to Fe oxide surface compared to that in other soil types.

4.6. Conclusion

This experiment confirms our hypothesis that the Fe oxide amendment can adsorb DOC and reduce the release of organic carbon from soil, regardless of soil types. Comparisons for the amount DOC reduction between different soil types support that soil properties (especially soil texture, soil organic matter and pH) play an important role on DOC adsorption by Fe oxides. Therefore it is justified to run a further experiment with an aim to investigate the effect of Fe oxide amendment on storing organic carbon in soil aggregates (Chapter 5).

4.5. References

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Chapter 5

Effect of Fe oxide in improving soil aggregation and stabilising soil organic carbon

5.1. Introduction

It has been well established that poorly crystalline Fe oxides affect soil aggregation and long term SOC stabilisation (Kleber et al., 2005; Kögel-Knabner et al., 2008). The possible mechanisms of soil organic carbon (SOC) stabilisation by Fe oxide were discussed in Chapter 2 (Section 2.6.). Formation of organo-mineral complex is one of the dominant mechanisms for long term SOC preservation. Research suggests that the effect of poorly crystalline Fe oxide on SOC preservation is further accelerated by the addition of organic amendments in soils (Wen et al., 2019). In this Chapter, we aimed to investigate the effect of poorly crystalline Fe oxides, organic matter (OM), and the interactive effect of poorly crystalline Fe oxides + OM on soil aggregate formation and organic carbon stabilisation.

The main hypotheses that we tested in this experiment were:

1. Fe oxide would increase soil aggregate formation both at macro-aggregate and micro-aggregate level.
2. Fe oxide will reduce the amount of easily decomposable carbon and increase the amount of stable organic carbon.
3. The combined effect of Fe oxide + organic matter would increase aggregation and stable organic carbon more than any other treatment.

5.2. Methods

We carried out a pot experiment where wheat plants were grown for 8 weeks. Soil samples for the pot experiment were collected from the agricultural land in the Nafferton ecological farm, University of Newcastle. Sample collection, and preparation methods were described in Chapter 3, Section 3.2. The basic properties of this soil and sampling location were presented in Chapter 3, Table 3-1. Wheat (*Triticum aestivum*) seeds were bought from the cultivar named Skyfall.

Approximately 350g of air-dry soil was transferred into each plastic pot. Four different levels of treatments were applied: 1) Control (wheat plants) 2) Fe oxides (wheat plants + four different percentages of Fe oxides) 3) OM (wheat plants + four different levels of OM) 4) Fe oxides + OM (wheat plants + four different percentages of Fe oxides in combination with four different level of OM). The experiment followed a completely randomised block design with three replicates for each treatment. For all cases, wheat seedlings were planted after the addition of any soil amendments. OM (sewage sludge) was added at a level based on maximum permitted additions of N (250 kg/ha) under UK sludge legislation (Tamsyn Birgitta Wilshire Kiss, Postgraduate researcher, Department of Environment and Geography, University of York, 2019). In this experiment, OM (sewage sludge) was applied to give a concentration of 0, 1.5, 3, 6, and 12 tons per hectare soil. Fe oxides (ochres) were added at a weight percentage of 0%, 0.5 %, 1 %, 2 %, and 4% of soil (wet weight of ochres and air-dry weight of soil). Soil pH, extractable Fe, and the mass of water-stable aggregate fractions were determined in all the amended soil samples prior to the plant growth. Detailed procedures for the measurement of soil pH, extractable Fe, and the mass of water-stable aggregate fractions are available in Chapter 3 (Section 3.3; Section 3.8. and Section 3.6. respectively). Wheat seeds were sown on tissue paper and watered every day. After one week, all the seeds had germinated. Three wheat seedlings were transferred at a depth of 1 cm in each pot. Plants were grown under natural light for eight weeks. An adequate level of soil moisture was maintained by watering the plants. Approximately 80-100 ml of deionised water was added in each treatment pot twice a week based on the moisture content of the soil, ochres and sewage sludge (10 g of each air-dry soil, moist ochres and sewage sludge were dried in the oven at 105° C and the moisture content was determined by the mass difference between the moist weight for each of the soil, ochres, sewage sludge and the oven-dry weight for each of the soil, ochres and sewage sludge). After eight weeks, plants were harvested and the dry matter biomasses of the plants were measured. Soil from each treatment pot was homogenised and immediately used for soil respiration measurement. The detailed procedure of soil respiration measurement is described in Chapter 3, Section 3.7. The rest of the soil samples were air-dried, bagged and labelled for the analysis of soil pH, extractable Fe, and the mass of water-stable aggregate fractions. The masses of aggregate fractions before and after the experimental period were compared and the concentration of organic carbon was measured for the aggregate fractions which significantly differed between the beginning and the end of the experiment. Detailed procedures for the measurement of organic carbon in solid aggregate fractions and the measurement of dissolved organic carbon were described in Chapter 3, Section 3.6.

5.3. Results

5.3.1. Changes in soil respiration

We measured soil respiration in order to see whether Fe oxide or OM treatment triggered any changes in soil microbial activity. The results for the amount of oxygen decline (%) per minute in the soil are presented in Figure 5-1. There were no significant changes in the amount of oxygen decline (%) per minute due to the individual effect of Fe oxide or OM treatment ($P \geq 0.05$) (Table 5-1.). However, the interactive effect of Fe oxide + OM was significant ($P=0.025$).

All pairwise multiple comparisons found that the respiration rate was significantly higher ($P \leq 0.05$) for the 12 t/ha OM treatment than 0, 1.5, 3, and 6 t/ha OM within 0.5% Fe oxide treatment. 0.5% Fe oxide had significantly higher effect ($P \leq 0.05$) than 0, 1, 2, 4% Fe oxide treatment within 12 t/ha OM level (Figure 5-1). This result indicates that higher amounts of OM with a very small amount of Fe oxide amendment creates a higher microbial oxygen consumption rate. There was no significant interaction between any other combinations of Fe oxide + OM treatment.

Table 5- 1: Two-way analysis of variance results of the effects of Fe oxide and organic matter on the soil respiration rate (data passed the normality test P = 0.297, and equal variance test P= 0.910).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	0.251	0.0628	0.184	0.946
OM	4	2.137	0.534	1.562	0.199
Fe oxide + OM	16	11.398	0.712	2.083	0.025

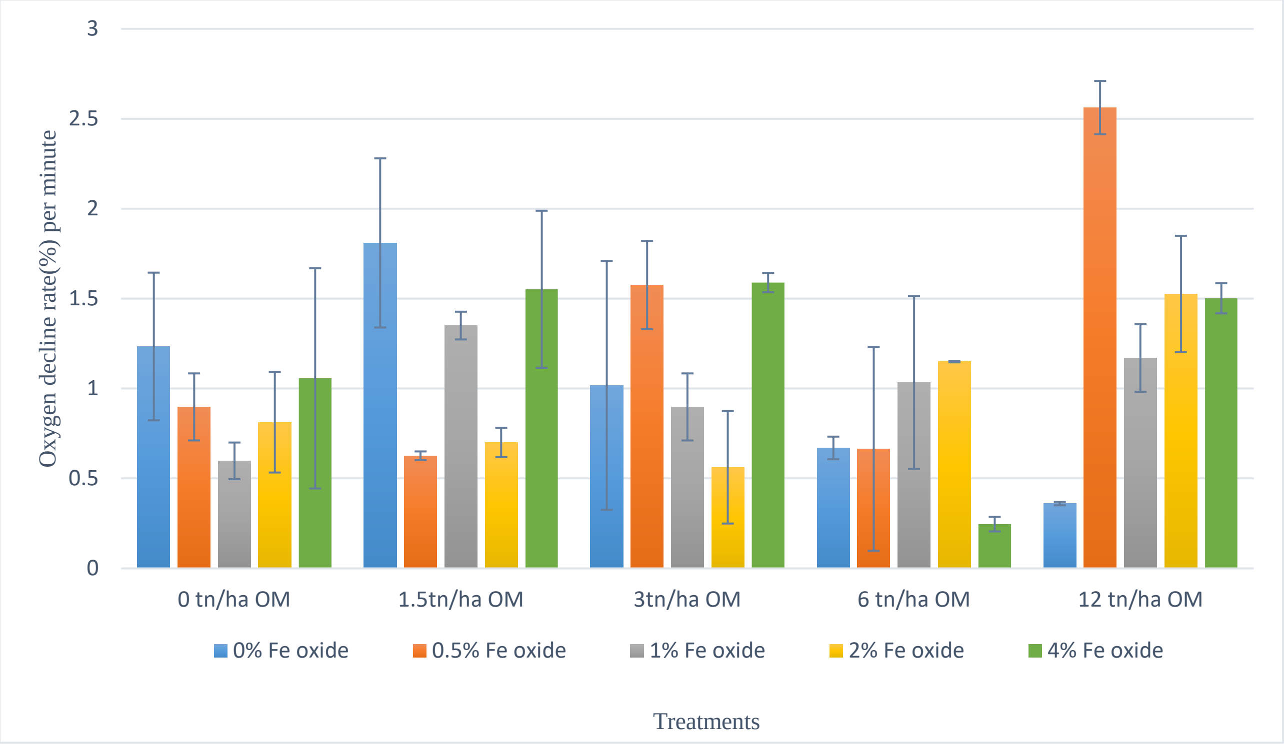


Figure 5- 1: Oxygen decline rate (%) per minute in soil due to the different levels of Fe oxide, OM, and Fe oxide+OM treatments (n= 3, error bars indicate standard deviation).

5.3.2. Wheat plant dry weight biomass

The dry matter biomasses of the wheat plants are presented in Figure 5-2. Wheat plants dry matter biomass (g) did not differ significantly in Fe oxide treatments whereas there was a significant difference in plant biomasses due to OM treatments (Figure 5-2, Table 5-2.). The interactive effects of Fe oxide + OM treatments were not significant.

Pairwise multiple comparisons indicated that dry matter biomass of wheat plant increased significantly ($P<0.05$) between each level of OM addition. The greatest value of wheat plants dry biomass was found at 12 t/ha OM treatment.

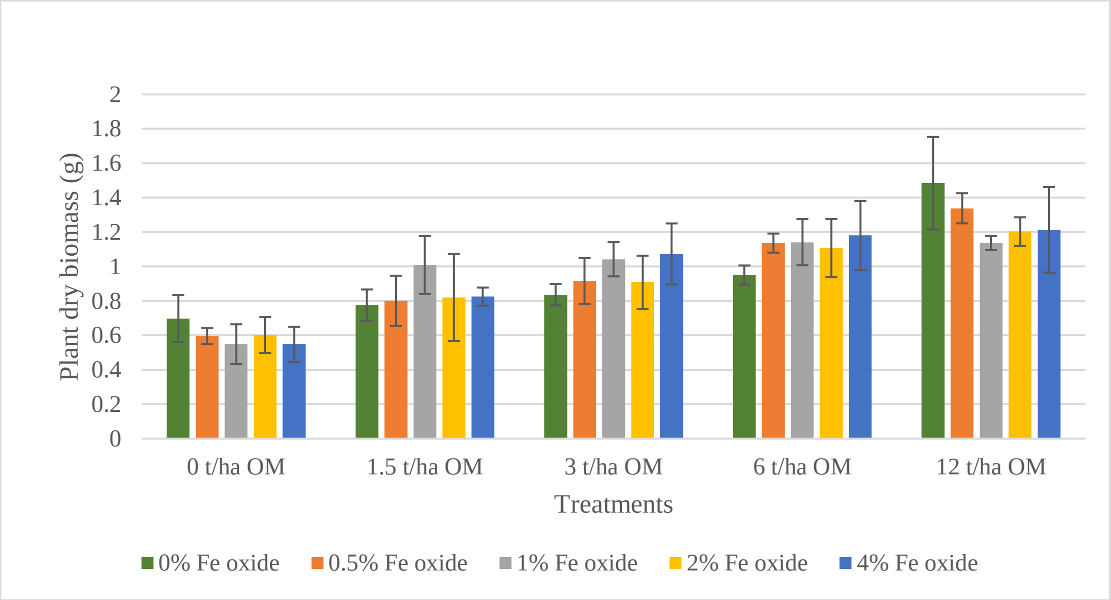


Figure 5- 2: Wheat plants dry matter biomasses (g) in different Fe oxide, OM and Fe oxide + OM treatments (n=3, error bar indicates standard deviation).

Table 5- 2: Two-way analysis of variance results of the effects of Fe oxide and organic matter on wheat plant dry matter biomass (data passed the normality test P =0.172, and the equal variance test P= 0.915).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	0.0203	0.00507	0.249	0.909
OM	4	3.944	0.986	48.394	<0.001
Fe oxide + OM	16	0.570	0.0356	1.749	0.067

5.3.3. Changes in soil pH

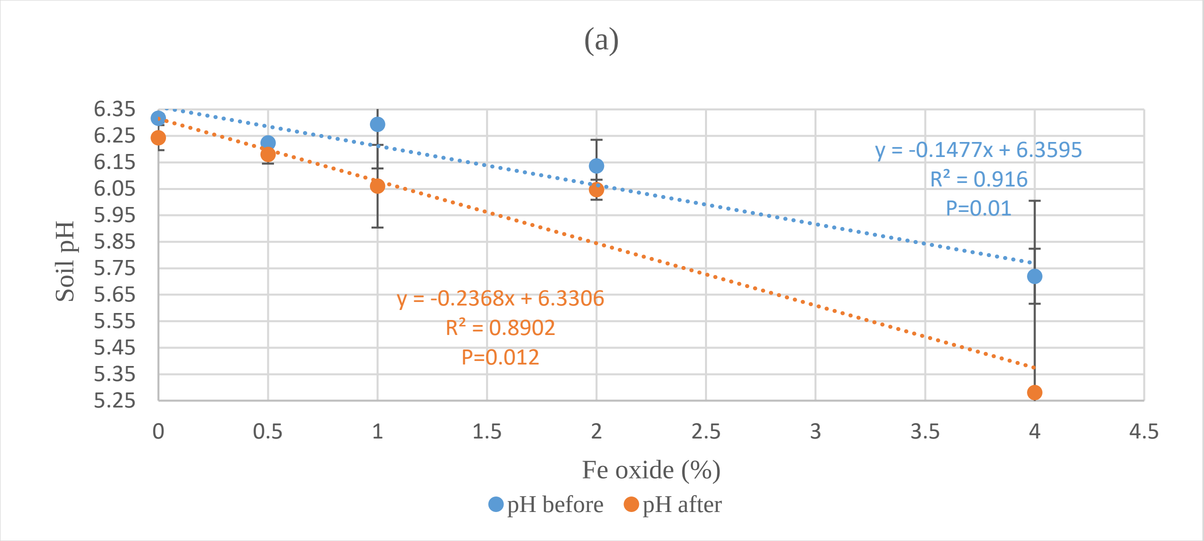
Soil pH was measured at the beginning and at the end of the experiment. Changes in soil pH value due to different levels of Fe oxide, OM, and Fe oxide + OM treatments are shown in Figure 5-3. Soil pH decreased significantly (P< 0.001) over the experimental period regardless of any treatments. There was a significant effect (P< 0.001) of Fe oxide on soil pH (Table 5-3.) whereas there was no significant effect of OM. The interaction of Fe oxide + OM was significant (P= 0.012). The interactive effect of Fe oxide + time, OM + time, and Fe oxide + OM + Time were not significant on the soil pH level (Table 5-3.).

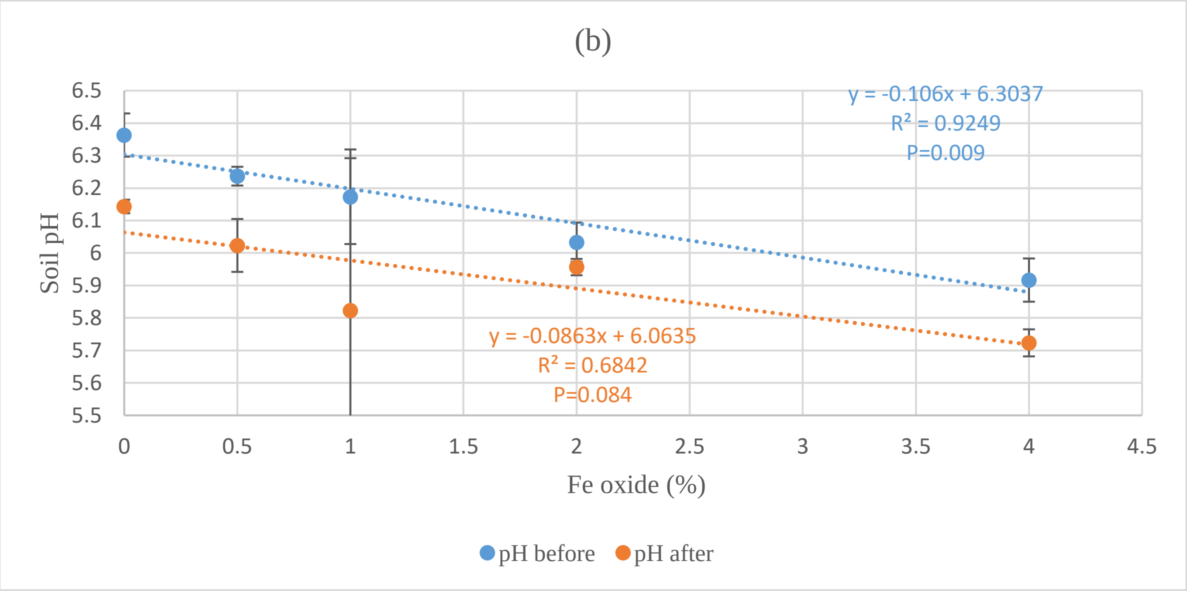
Pairwise multiple comparisons showed that soil pH was the lowest at 4% Fe oxide treatment compared to any other Fe oxide treatment. For all the OM treatments soil pH values were not significantly different within 0, 0.5, 1, and 2 % Fe oxide level. We observed that the combined effect of OM (1.5, 3, 6 and 12 t/ha) with 4% Fe oxide treatment showed significantly lower pH compared to the individual effect of OM. Similarly, 1.5, 6 and 12 t/ha OM with 2% Fe oxide treatment had significantly lower soil pH compared to the individual effect of OM. These results support that the addition of Fe oxide reduces soil pH.

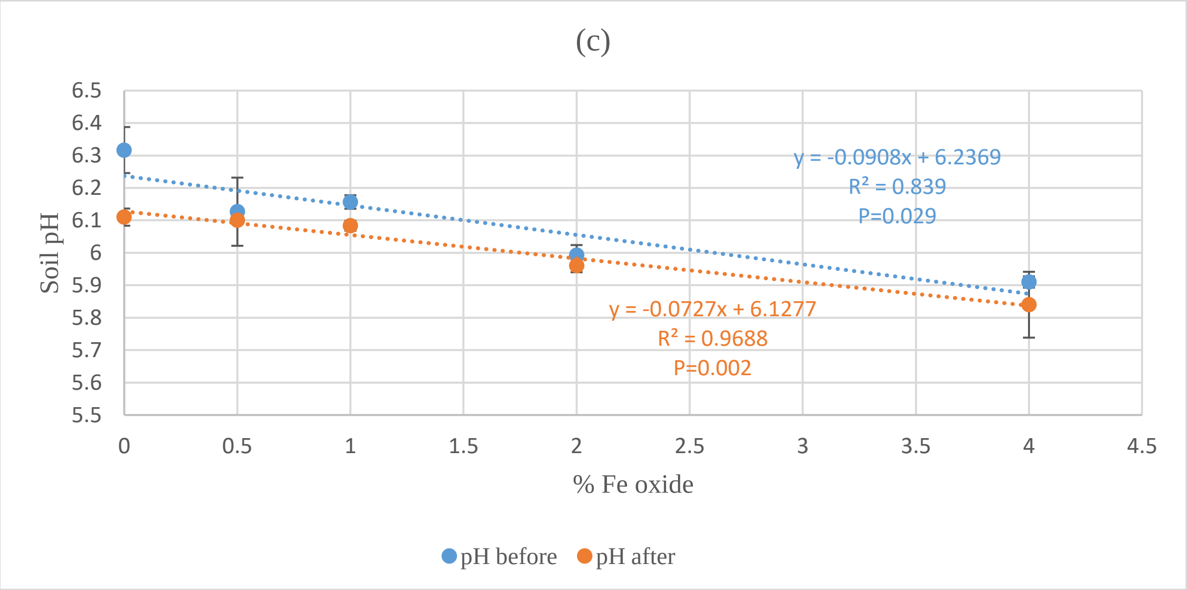
pH in the 0.5 % Fe oxide treatments with 1.5, 6, and 12 t/ha OM were greater than for the 4% Fe oxide treatment with the same levels of OM (1.5,6, and 12 t/ha) treatments. This result indicates a higher rate of Fe oxide addition lowers the soil pH. However, the addition of any rate of OM amendment with Fe oxide raised the soil pH level.

Table 5- 3: Three-way analysis of variance results of the effects of Fe oxide, organic matter, and time on soil pH (data did not pass the normality test P <0.05, but passed the equal variance test P= 0.108).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	4.183	1.046	44.377	<0.001
OM	4	0.0288	0.00721	0.306	0.873
Time	1	0.741	0.741	31.425	<0.001
Fe oxide + OM	16	0.809	0.0505	2.145	0.012
Fe oxide + Time	4	0.133	0.0332	1.410	0.236
OM + Time	4	0.0865	0.0216	0.918	0.457
Fe oxide + OM + Time	16	0.230	0.0144	0.610	0.869


(a)
Soil pH
6.35
6.25
6.15
6.05
5.95
5.85
5.75
5.65
5.55
5.45
5.35
5.25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Fe oxide (%)
y = -0.1477x + 6.3595
R² = 0.916
P=0.01
y = -0.2368x + 6.3306
R² = 0.8902
P=0.012
● pH before
● pH after


(b)
Soil pH
6.5
6.4
6.3
6.2
6.1
6
5.9
5.8
5.7
5.6
5.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Fe oxide (%)
y = -0.106x + 6.3037
R² = 0.9249
P=0.009
y = -0.0863x + 6.0635
R² = 0.6842
P=0.084
● pH before
● pH after


(c)
Soil pH
6.5
6.4
6.3
6.2
6.1
6
5.9
5.8
5.7
5.6
5.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
y = -0.0908x + 6.2369
R² = 0.839
P=0.029
y = -0.0727x + 6.1277
R² = 0.9688
P=0.002
● pH before
● pH after

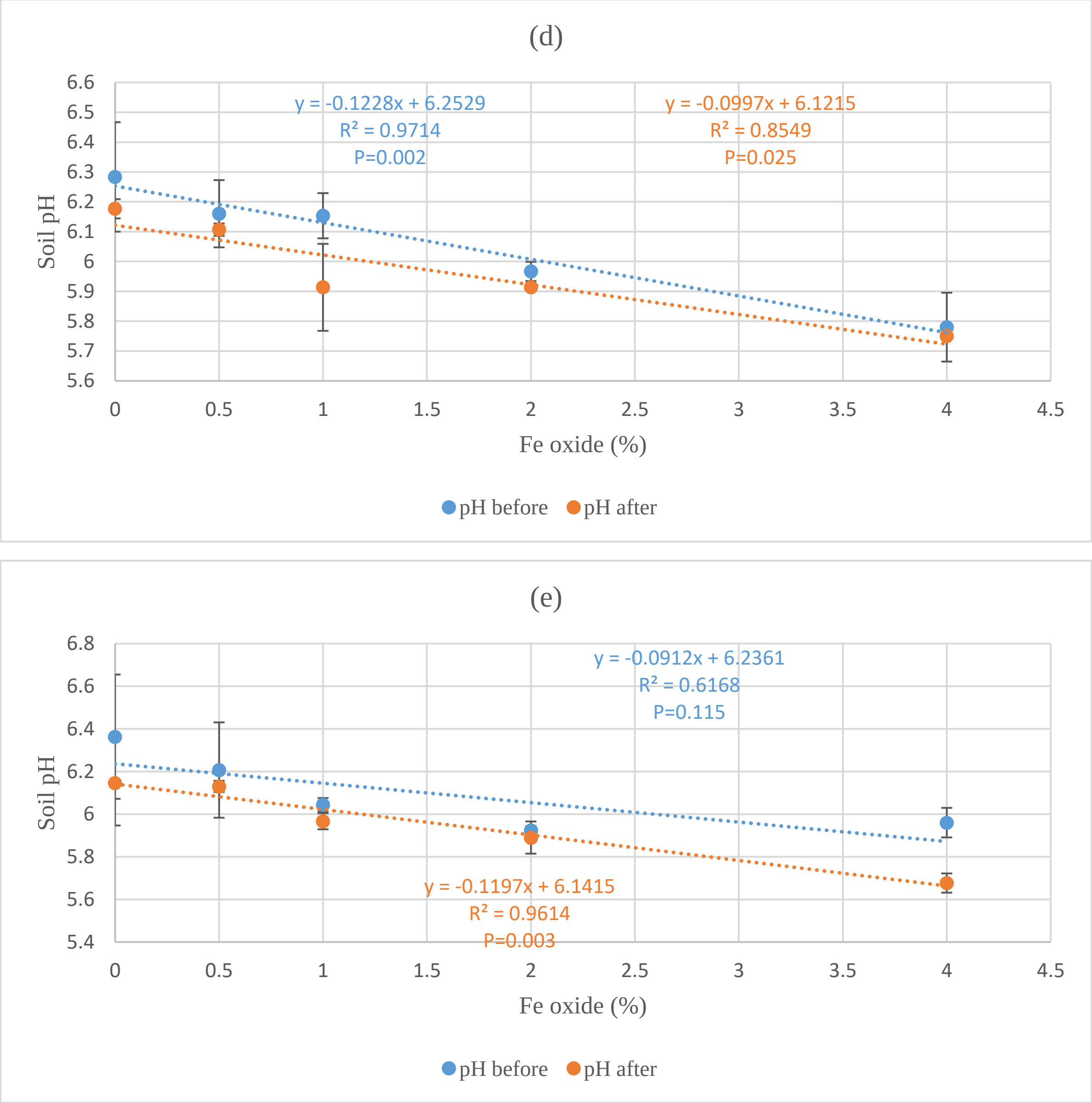


Figure 5- 3: Changes in soil pH over the experimental period due to Fe oxide (%) treatments at (a) 0 t/ha OM (b) 1.5 t/ha OM (c) 3 t/ha OM (d) 6 t/ha OM (e) 12 t/ha OM (n=3, error bars indicate standard deviation).

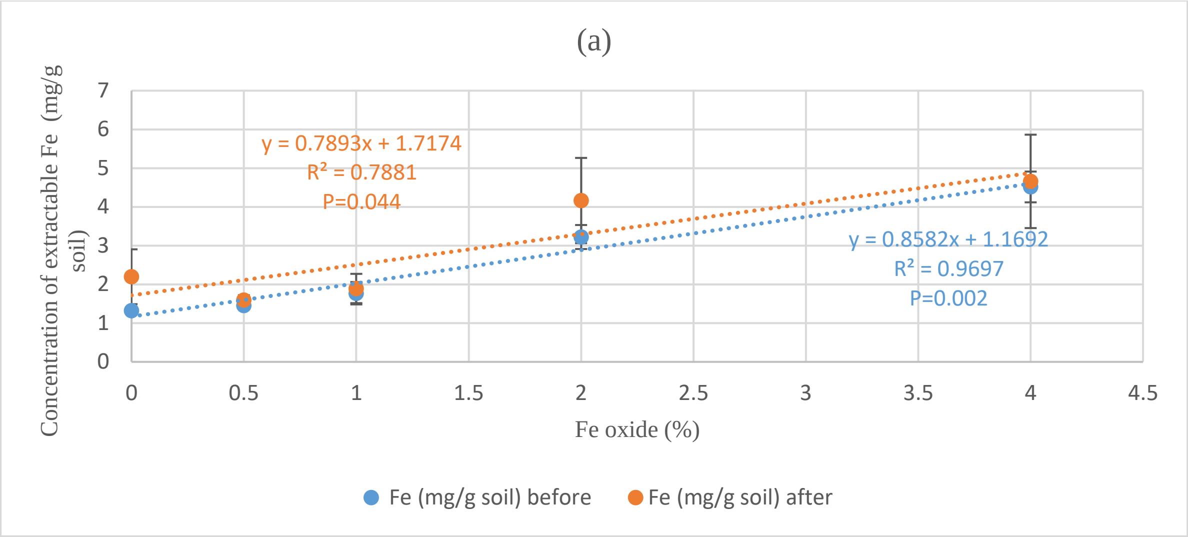
5.3.4. Changes in the concentration of dithionate extractable Fe (Crystalline plus poorly crystalline Fe)

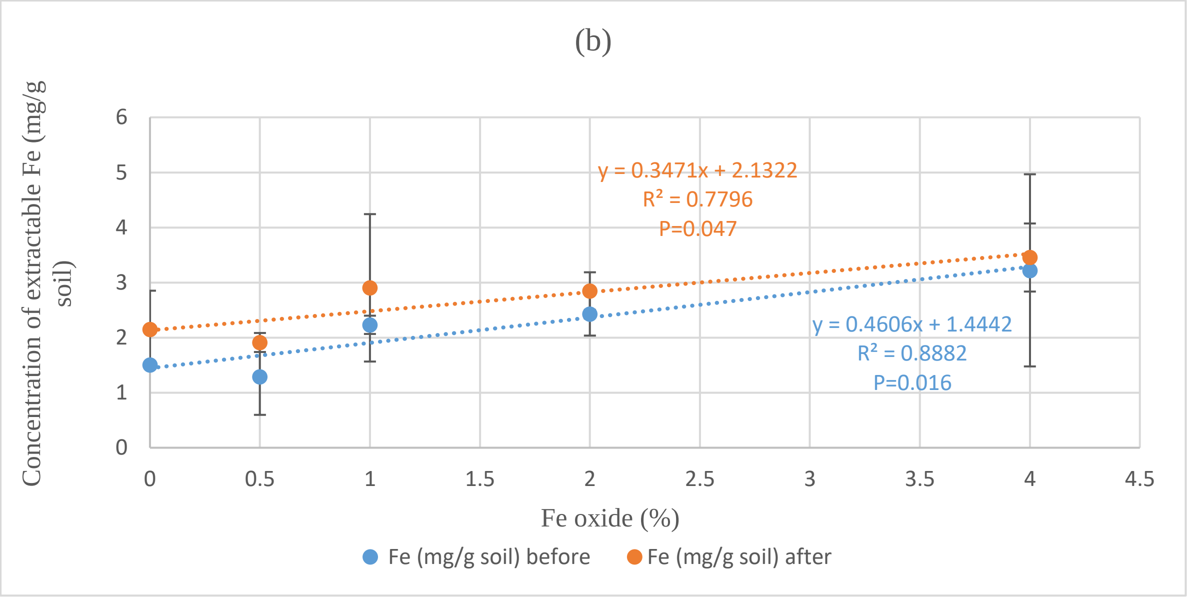
Changes in the concentration of dithionate extractable Fe over the experimental period are shown in Figure 5-4. The concentration of extractable Fe was significantly higher ($P< 0.001$) at the end of the experiment regardless of any treatments. (Figure 5-4). There was a significant effect ($P<0.05$) of Fe oxide treatments on the concentration of extractable Fe but the effect of OM was not significant ($P>0.05$). The combined effect of Fe oxide + OM was significant (Table 5-4.).

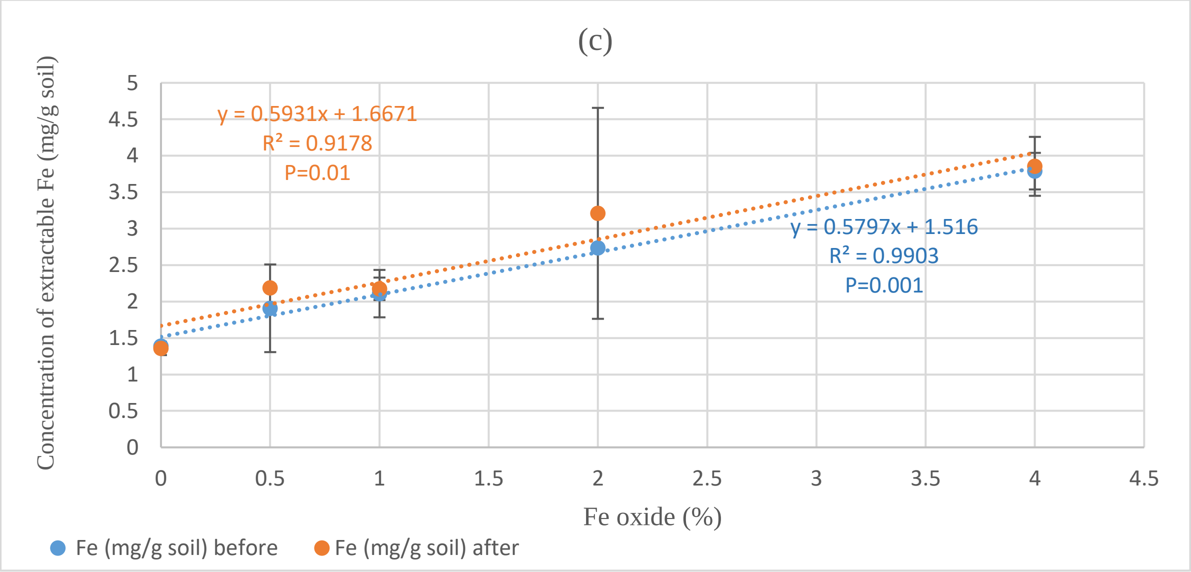
Pairwise multiple comparisons showed that the concentration of extractable Fe significantly ($P<0.05$) increased with the increase in each level of Fe oxide treatment. For each of the 0, 0.5, and 1% Fe oxide treatments the amount of extractable Fe was not significantly different at different OM levels. For 2% and 4% Fe oxide treatments, there was more extractable Fe at no OM level compared to their interaction with 1.5 t/ha OM treatment. The interaction of 3, 6, and 12 t/ha OM with 4% Fe oxide level caused a significantly greater concentration of extractable Fe compared to their interactions with 0, 0.5 and 1% Fe oxide. The same observations were found for the interactions of 6 and 12 t/ha OM treatment with 2% Fe oxide. In all cases of significant interactions between Fe oxide and OM, the results showed that the concentration of extractable Fe was higher within the interaction of Fe oxide + OM compared to that of only OM treatments. The addition of a higher dose of Fe oxide with any level of OM produces more extractable Fe. This result supports that a higher level of Fe oxide treatment produced more extractable Fe regardless of what level of OM was given.

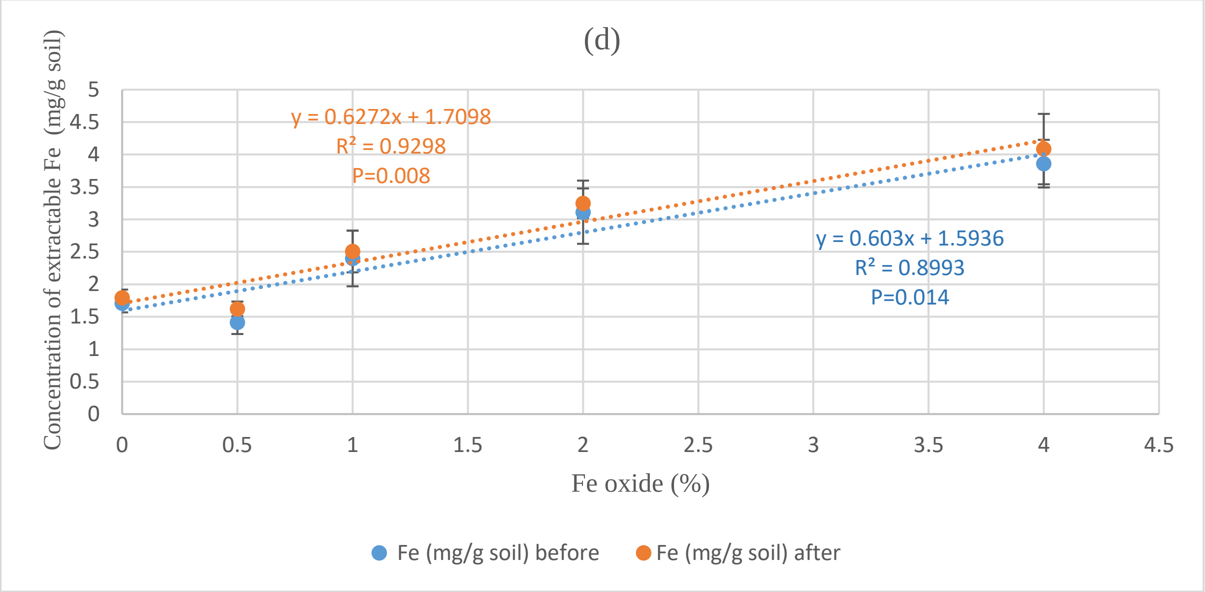
Table 5- 4: Three-way analysis of variance results of the effects of Fe oxide, organic matter, and time on the concentration of dithionate extractable Fe (data did not pass the normality test $P <0.05$, but passed the equal variance test $P= 0.259$).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	119.565	29.891	87.012	<0.001
OM	4	1.523	0.381	1.108	0.357
Time	1	4.646	4.646	13.523	<0.001
Fe oxide + OM	16	11.930	0.746	2.171	0.011
Fe oxide + Time	4	0.355	0.0889	0.259	0.904
OM +Time	4	0.926	0.231	0.674	0.612
Fe oxide + OM + Time	16	1.979	0.124	0.360	0.988


(a)
Concentration of extractable Fe (mg/g soil)
7
6
5
4
3
2
1
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Fe oxide (%)
y = 0.7893x + 1.7174
R² = 0.7881
P=0.044
y = 0.8582x + 1.1692
R² = 0.9697
P=0.002
● Fe (mg/g soil) before
● Fe (mg/g soil) after


(b)
Concentration of extractable Fe (mg/g soil)
6
5
4
3
2
1
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Fe oxide (%)
y = 0.3471x + 2.1322
R² = 0.7796
P=0.047
y = 0.4606x + 1.4442
R² = 0.8882
P=0.016
● Fe (mg/g soil) before
● Fe (mg/g soil) after


(c)
Concentration of extractable Fe (mg/g soil)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Fe oxide (%)
y = 0.5931x + 1.6671
R² = 0.9178
P=0.01
y = 0.5797x + 1.516
R² = 0.9903
P=0.001
● Fe (mg/g soil) before
● Fe (mg/g soil) after



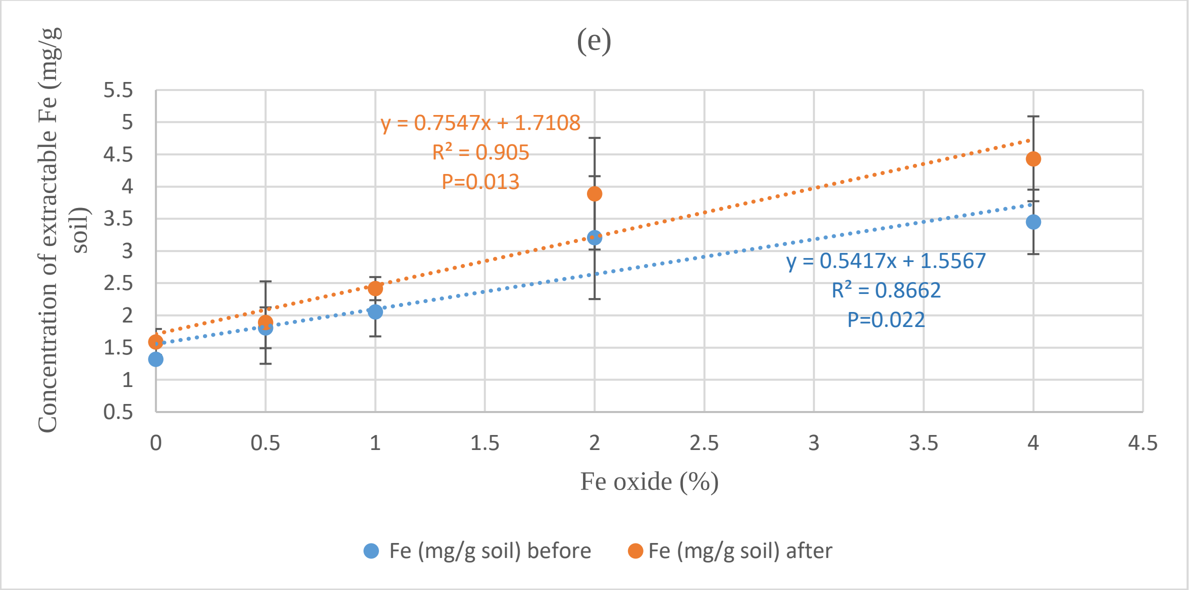


Figure 5- 4: Changes in the concentration of extractable Fe (mg/g soil) over the experimental time due to different levels of Fe oxide (%) treatments within (a) 0 t/ha OM (b) 1.5 t/ha OM (c) 3 t/ha OM (d) 6 t/ha OM (e) 12 t/ha OM (n=3, error bars indicate standard deviation).

5.3.5. Changes in soil aggregate fractionations

Soil samples were fractionated into five different categories. We compared the changes in mass of each solid fraction over the incubation period. Then we analysed the concentration of organic carbon in fractions on the basis of significant changes in masses of soil fractions due to the effect of OM, Fe oxide, and the interaction of Fe oxide +OM over time. Results for the masses of all the fractions followed by carbon content in soil fractions are presented below:

5.3.5.1. Changes in masses of sand and stable aggregates (obtained from >250 µm fraction)

Changes in masses of sand and stable aggregate (S+A) fractions over the incubation period are shown in Figure 5-5. A three-way analysis of variance showed that the individual effect of Fe oxide and organic matter was significant (P< 0.001 and P= 0.007, respectively) on the masses of (S+A) fractions (Table 5-5.). There was a significant increase in the masses of (S+A) fractions over the duration of experimental time (P<0.001). The masses of (S+A) fractions significantly differed within the interactive effect of Fe oxide + time whereas the interaction of OM+ time was not significant. A significant difference in masses was observed for the interaction of Fe oxide + OM (P= 0.002) but there was no significant difference between the interactions of Fe oxide + organic matter + time (P= 0.052).

Table 5- 5: Three-way analysis of variance results of the effects of Fe oxide and Organic matter and time on the masses of sand and stable aggregate fractions (data did not pass the normality test P<0.05, passed equal variance test P=0.202).

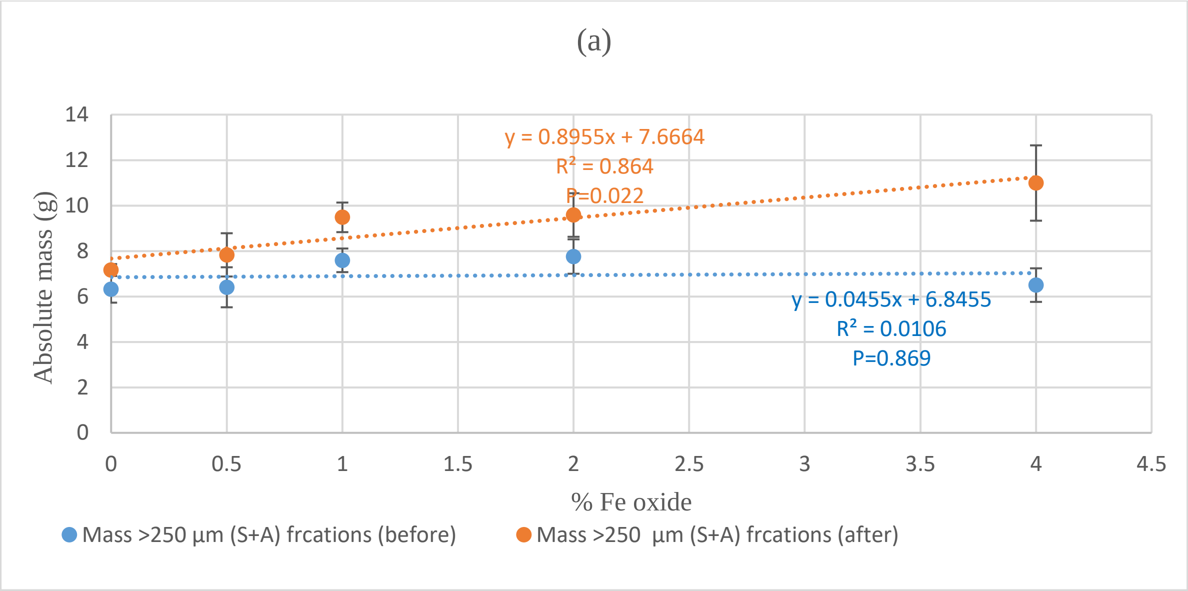
Source of Variation	DF	SS	MS	F	P
Fe oxide	4	56.328	14.082	8.040	<0.001
OM	4	22.181	5.545	3.166	0.017
Time	1	177.747	177.747	101.487	<0.001
Fe oxide + OM	16	61.002	3.813	2.177	0.010
Fe oxide + Time	4	30.011	7.503	4.284	0.003
OM + Time	4	1.071	0.268	0.153	0.961
Fe oxide + OM + Time	16	46.101	2.881	1.645	0.071

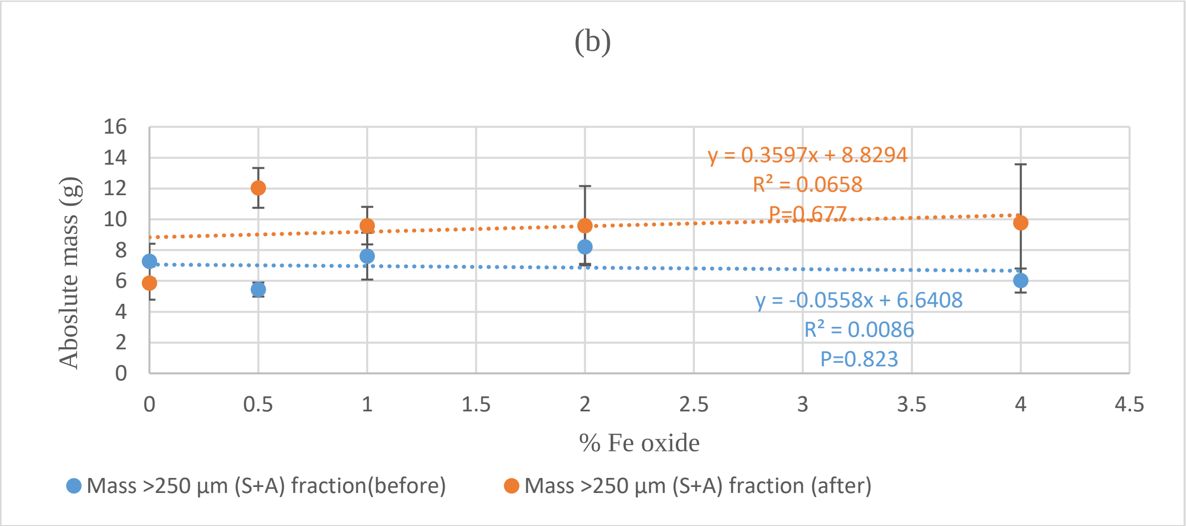
All pairwise multiple comparisons showed that at the end of the experiment the masses of (S+A) fractions were significantly higher (p<0.001) for all the Fe oxide treatments compared to that of the start of the experiment. Among all the different levels of Fe oxide treatments, 2% Fe oxide treatment showed the largest mass of S+A fraction at the end of the experiment.

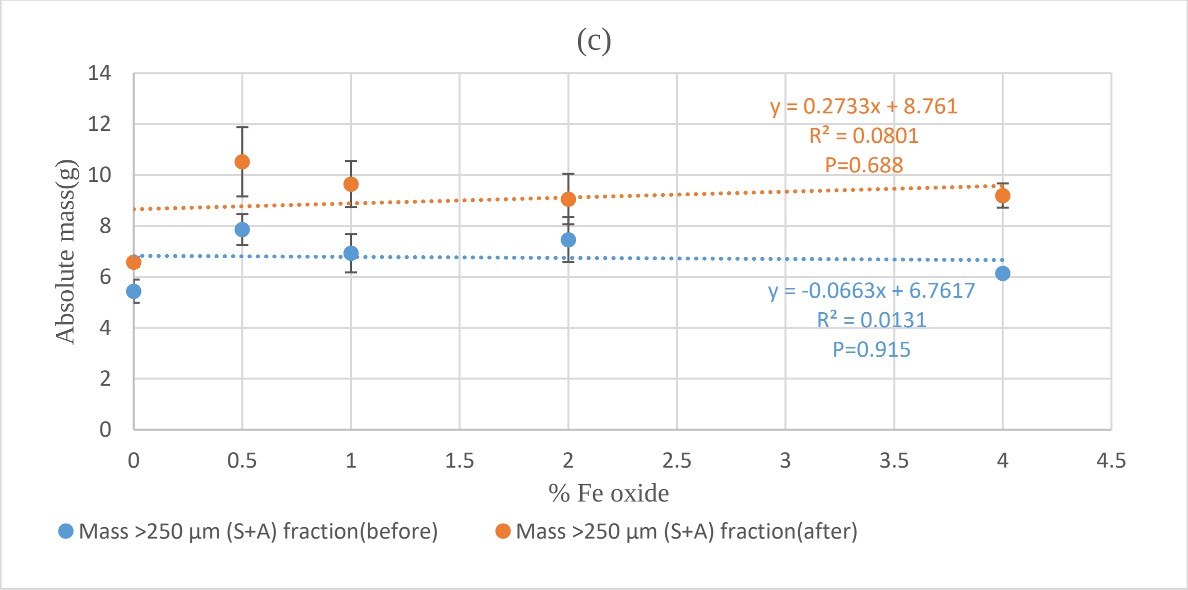
The masses of S+A fractions increased with the increase in OM (no Fe oxide) treatments.

Comparisons for the Fe oxide + OM interactions showed significant interactions in many different combinations. The masses of (S+A) fractions were significantly higher ($P \leq 0.05$) at 0.5% Fe oxide + 1.5 t/ha OM and 0.5% Fe oxide + 3 t/ha OM treatment compared to the individual treatment of 0.5% Fe oxide, 1.5 t/ha OM and 3 t/ha OM. Similarly, the combined effect of 2% Fe oxide +1.5 t/ha OM and 2% Fe oxide + 3 t/ha OM were significantly higher than the individual effect of 1.5 t/ha OM and 3 t/ha OM. This result indicates that the interactive effect of Fe oxide +OM causes a greater increase in masses (S+A) of fractions than that of their individual effect. The masses of S+A fractions were significantly higher ($P= 0.019$) for 12 t/ha OM than that of 3t/ha OM within 2% Fe oxide level. This result supports that keeping the Fe oxide level constant, higher levels of OM addition can cause a greater increase in masses of S+A fractions. Comparisons for the effect of different Fe oxide treatments within 12 t/ha OM showed that 2% Fe oxide with 12 t/ha OM had significantly greater mass than 0.5% and 1% Fe oxide treatments. This indicates for a particular level of organic matter increasing Fe oxide levels caused an increase in masses of S+A fractions. Therefore, our result indicates within the combined effect of Fe oxide +OM, both Fe oxide and OM equally contributed to cause an increase in masses of S+A fractions. There was no significant difference between the OM treatments at 1% and 4% Fe oxide level. There was no significant difference between the Fe oxide treatments at 6 t/ha and 0 t/ha OM level.

Overall both Fe oxide and OM addition caused an increase in masses of S+A fraction. The higher amount of OM addition caused higher masses of S+A fractions. The combined effect of Fe oxide +OM caused greater masses of S+A fraction than that of their individual effect. Within different combinations of Fe oxide +OM treatments, both Fe oxide and OM equally contributed to the increase in masses of S+A fractions. However, the masses of S+A fractions did not significantly differ due to the effect of OM and its combination with Fe oxide over the incubation period. Only Fe oxide treatment caused significantly higher masses of S+A fractions by the end of the experiment compared to the beginning.


(a)
y = 0.8955x + 7.6664
R² = 0.864
P=0.022
y = 0.0455x + 6.8455
R² = 0.0106
P=0.869
Absolute mass (g)
0
2
4
6
8
10
12
14
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
● Mass >250 µm (S+A) frcations (before)
● Mass >250 µm (S+A) frcations (after)


(b)
y = 0.3597x + 8.8294
R² = 0.0658
P=0.677
y = -0.0558x + 6.6408
R² = 0.0086
P=0.823
Aboslute mass (g)
0
2
4
6
8
10
12
14
16
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
● Mass >250 µm (S+A) fraction(before)
● Mass >250 µm (S+A) fraction (after)


(c)
y = 0.2733x + 8.761
R² = 0.0801
P=0.688
y = -0.0663x + 6.7617
R² = 0.0131
P=0.915
Absolute mass(g)
0
2
4
6
8
10
12
14
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
● Mass >250 µm (S+A) fraction(before)
● Mass >250 µm (S+A) fraction(after)

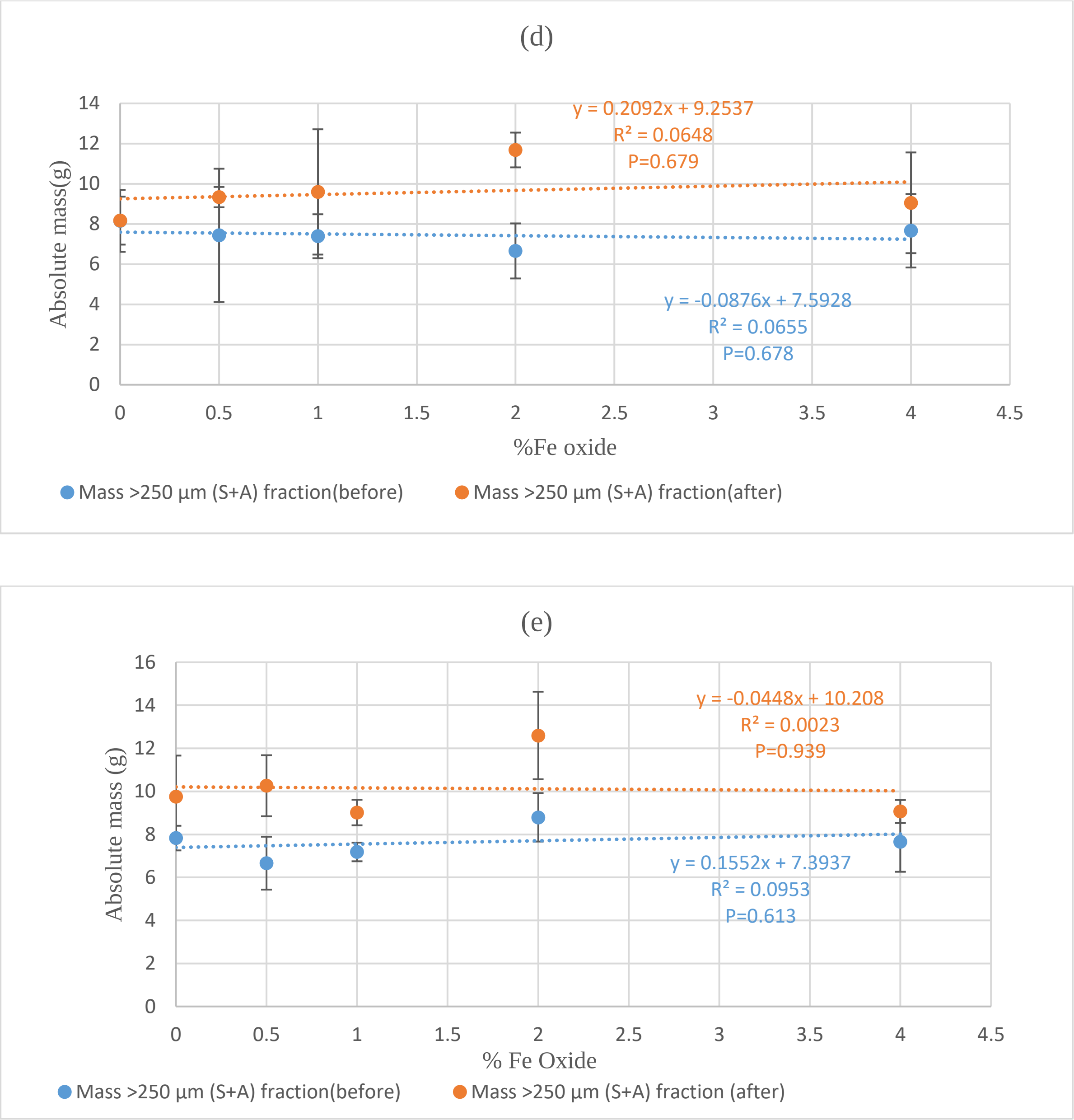


Figure 5- 5: Changes in masses of (S+A) fractions (>250 µm) over the experimental time due to different level of Fe oxide (%) treatments at (a) 0 t/ha OM (b) 1.5 t/ha OM (c) 3 t/ha OM (d) 6t/ha OM and (e) 12 t/ha OM (n=3, error bars indicate standard deviation).

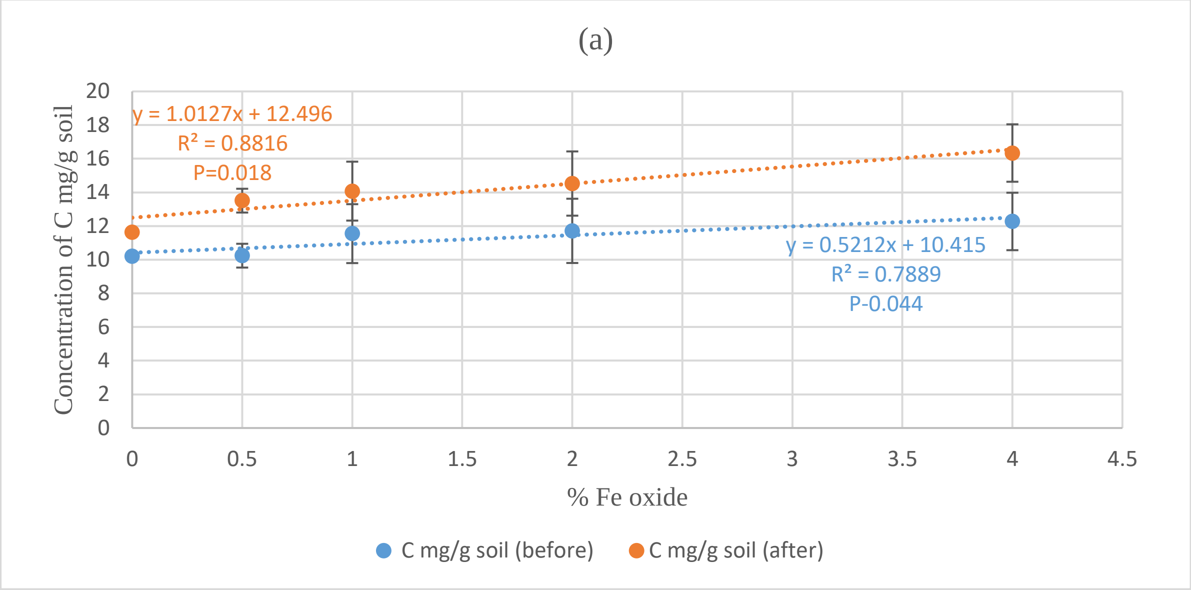
5.3.5.2. Concentration of organic C (mg/g soil) in S+A fractions (obtained from >250 µm fraction)

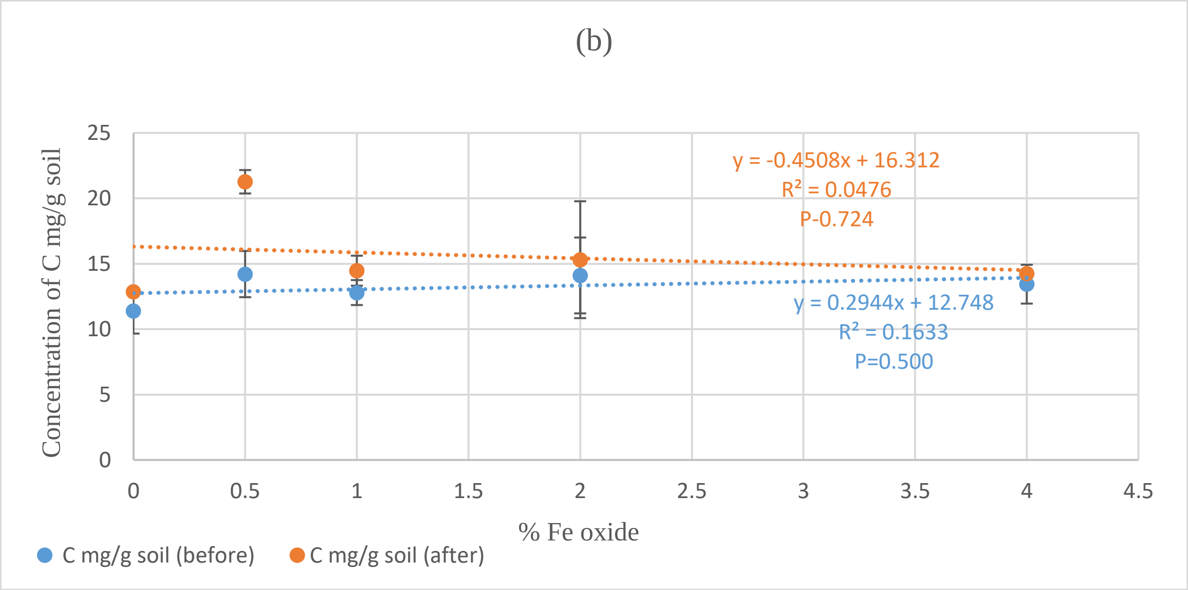
Changes in the concentration of organic carbon (mg/g soil) in the sand and stable aggregate (S+A) fractions (obtained from >250 µm fraction) over the experimental period are presented in Figure 5-6. A three-way analysis of variance (ANOVA) test found that the individual effect of Fe oxide (%), time, and organic matter (t/ha) on the concentration of organic C (mg/g soil) in S+A fractions was significant ($P \leq 0.05$). There was also a significant interaction between Fe oxide + OM ($P=0.016$) and that of OM + time ($P= 0.045$). There was no significant interaction between Fe oxide + time ($P=0.065$) but a significant three-way interaction of Fe oxide + OM+ time was observed (Table 5-6.). Therefore, the individual effect of each factor was not consistent at all combinations of two other factors.

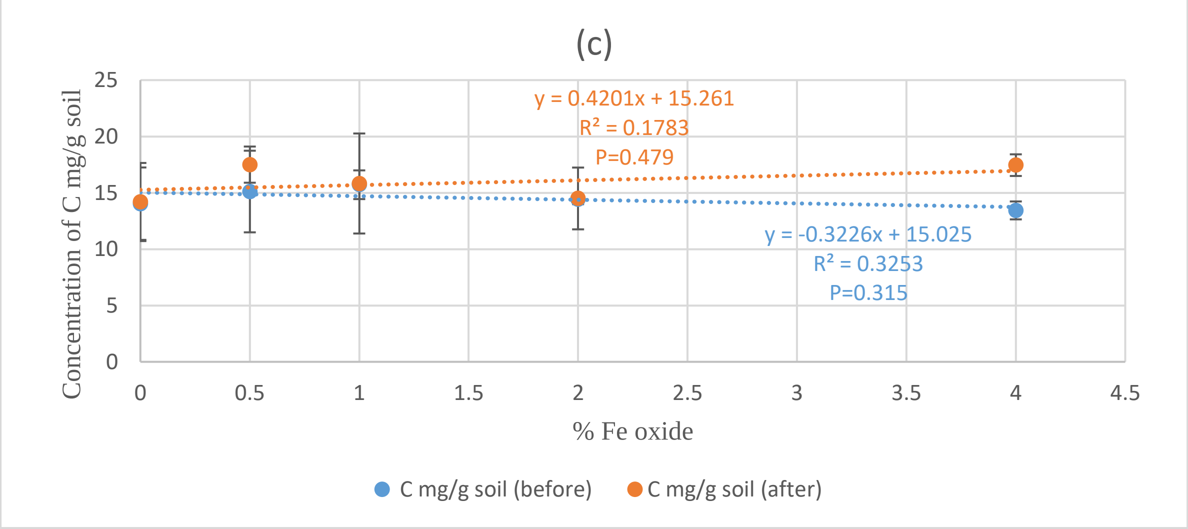
All pairwise multiple comparisons between different treatments showed many significant interactions between the three factors (% Fe oxide, OM, and Time).

Table 5- 6: Three-way analysis of variance results of the effects of Fe oxide and Organic matter and time on the concentration of organic carbon in S+A fractions obtained from >250 µm fraction (data did not pass the normality test $P<0.05$, passed equal variance test $P=0.057$).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	108.079	27.020	5.507	<0.001
OM	4	334.212	83.553	17.029	<0.001
Time	1	123.302	123.302	25.130	<0.001
Fe oxide + OM	16	162.073	10.130	2.065	0.016
Fe oxide + Time	4	44.980	11.245	2.292	0.065
OM + Time	4	49.732	12.433	2.534	0.045
Fe oxide + OM + Time	16	142.560	8.910	1.816	0.039


(a)
Concentration of C mg/g soil
% Fe oxide
0
2
4
6
8
10
12
14
16
18
20
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
y = 1.0127x + 12.496
R² = 0.8816
P=0.018
y = 0.5212x + 10.415
R² = 0.7889
P-0.044
C mg/g soil (before)
C mg/g soil (after)


(b)
Concentration of C mg/g soil
% Fe oxide
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
y = -0.4508x + 16.312
R² = 0.0476
P-0.724
y = 0.2944x + 12.748
R² = 0.1633
P=0.500
C mg/g soil (before)
C mg/g soil (after)


(c)
Concentration of C mg/g soil
% Fe oxide
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
y = 0.4201x + 15.261
R² = 0.1783
P=0.479
y = -0.3226x + 15.025
R² = 0.3253
P=0.315
C mg/g soil (before)
C mg/g soil (after)

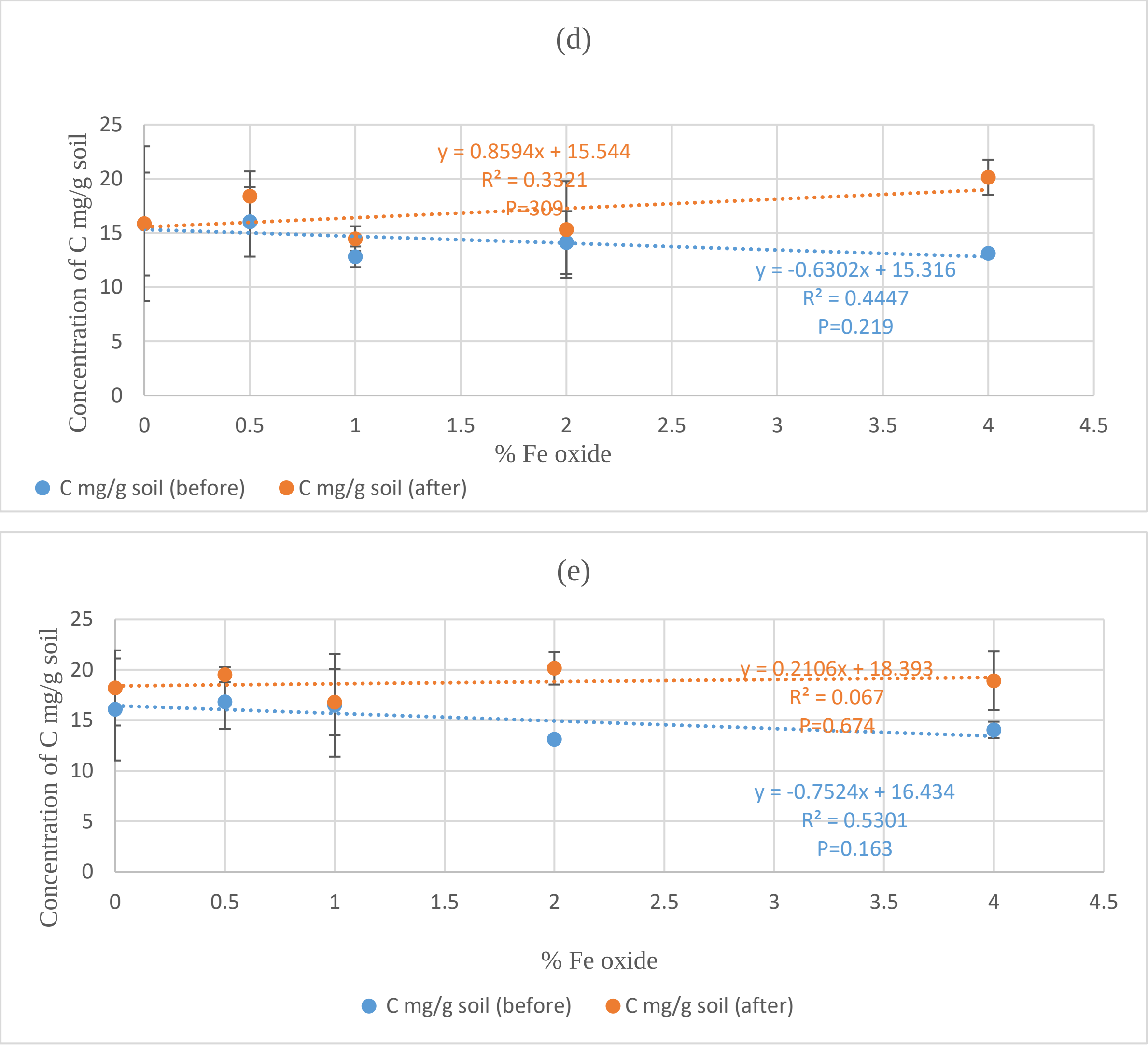


Figure 5- 6: Changes in the concentration of organic carbon in (S+A) fractions (>250 µm) over the experimental time within different levels of Fe oxide (%) treatments at (a) 0 t/ha OM (b) 1.5 t/ha OM (c) 3 t/ha OM (d) 6t/ha OM and (e) 12 t/ha OM (n=3, error bars indicate standard deviation).

The concentration of organic carbon (mg/g soil) was significantly higher after the experimental period than that of before the experiment regardless of any treatments.

The concentration of organic carbon in S+A fraction increased with increasing Fe oxide treatment (Figure 5-6, a). 0.5% Fe oxide treatment had significantly higher concentration of organic carbon than that of 0%, 1% and 2% Fe oxide (P=0.002, 0.007, and 0.002 respectively).

This result indicates that increasing the level of Fe oxide treatment did not cause an increase in carbon concentration in S+A fractions.

The concentration of organic C (mg/g soil) significantly increased with each level of OM addition. By the end of the experiment 12 t/ha OM caused the greatest increase in the concentration of organic carbon compared to that of other levels of OM treatments.

We did not observe any consistent trend of increase or decrease in organic carbon concentration in S+A fractions due to the combined effect of Fe oxide +OM. Pairwise multiple comparisons showed that there was a significant interaction ($P= 0.006$) between Fe oxide + OM + time at the end of the experiment but not before (0.095). It has been observed that at the end of the experiment 12 t/ha OM within 2% Fe oxide treatment had the greatest effect among all other interactions of OM (0, 1.5, 3, and 6 t/ha) with 2% Fe oxide ($P\leq0.05$). This result indicates increasing the level of OM with a particular level of Fe oxide caused an increase in the concentration of organic carbon in S+A fractions.

There were no significant differences between the effects of different levels of OM treatments evaluated within 1% and 4% Fe oxide level ($P=0.43$, $P=0.136$ respectively).

After 8 weeks the concentration of organic C (mg/g soil) was significantly higher for 0.5 % Fe oxide treatment within 1.5 t/ha OM than that of 0, 1, 2, 4% Fe oxide treatments ($P\leq0.05$). Similarly, 0.5% Fe oxide +3 t/ha OM had higher concentration of organic carbon than that of 2% Fe oxide+3 t/ha OM and 1% Fe oxide + 3 t/ha OM. This result indicates that at same level of OM increasing Fe oxide treatment did not cause any increase in the concentration of organic carbon. However, 4% Fe oxide+ 3 t/ha treatment showed a higher concentration of organic carbon than that of 2% Fe oxide+ 3 t/ha OM. This result indicates a contradictory statement of the previous one that at a particular level of OM increasing Fe oxide level did increase the concentration of organic carbon.

In order to find out a better insight of our result, we calculated the total amount of carbon (mg) in each fraction [mass of S+A fraction (g) multiplied by the carbon concentration (mg/g soil)] (Table 5-7.). A three-way analysis of variance (ANOVA) test found that there was a significant effect of Fe oxide + time on the amount of total organic carbon in S+A fractions (Table 5-8.). All pairwise multiple comparisons showed at the end of the experiment increasing Fe oxide treatments caused an increase in the amount of total organic carbon in S+A fractions and the greatest amount of total organic carbon was at 4% Fe oxide treatment.

Although increasing OM treatment showed an increase in total organic C in S+A fractions but the amount of total organic C did not differ between the start and the end of the experiment due to OM treatments.

We observed mostly similar results for the combined effect of Fe oxide + OM on both the concentration of organic C and total organic carbon in S+A fractions. The additional information was, pairwise multiple comparisons showed that 2% Fe oxide + 12 t/ha Fe oxide had significantly more total amount of organic carbon than that in 1% Fe oxide + 12 t/ha OM. This indicates adding more Fe oxide caused an increase in total organic carbon in S+A fractions.

Overall our result indicates the addition of both Fe oxide and OM helped to increase the concentration of organic carbon or the total amount of organic carbon in S+A fractions. Because of spatial variability, there was a lack of trend in the concentration of organic carbon or the total amount of organic carbon due to the interactive effect of Fe oxide +OM. Nevertheless, the comparisons within many different combinations of Fe oxide + OM treatment reveals that the concentration of organic carbon or total organic carbon increased significantly with increasing the level of Fe oxide in combination within the same level of OM treatment. Similarly, the concentration of organic carbon or total organic carbon increased significantly due to increasing the level of OM in combination within the same level of Fe oxide treatment. Therefore, it can be said that both Fe oxide and OM contributed to increasing the concentration of organic carbon in S+A fractions over the incubation period.

Table 5- 7: Mean masses of total organic carbon in (S+C) fractions (mg) before and after the experiment (n=3, ± standard deviation).

Treatments	Mass of total organic carbon in S+C (mg) fractions before 8 weeks	Mass of total organic carbon in S+C fractions (mg) after 8 weeks
Control	64.43±5.31	83.26±2.88
0.5% Fe oxide	65.90±15.46	105.90±15.80
1% Fe oxide	87.45±3.54	134.11±24.97
2% Fe oxide	90.97±10.62	139.54±26.73
4% Fe oxide	80.06±13.13	181.12±42.27
1.5 t/ha OM	82.35±11.67	75.54±14.35
3 t/ha OM	76.62±19.52	93.02±20.90
6 t/ha OM	130.15±50.26	134.86±80.67
12 t/ha OM	125.34±39.79	182.18±74.24
0.5 % Fe oxide + 1.5 t/ha OM	105.85±23.30	256.61±36.13
1% Fe oxide + 1.5 t/ha OM	96.57±14.20	139.61±28.39
2% Fe oxide + 1.5 t/ha OM	131.66±14.11	132.49±49.18
4% Fe oxide + 1.5 t/ha OM	81.15±14.87	139.48±54.46
0.5% Fe oxide + 3 t/ha OM	119.81±35.39	183.54±24.08
1% Fe oxide + 3 t/ha OM	108.20±5.5	109.17±12.47
2% Fe oxide + 3 t/ha OM	107.27±9.84	116.81±6.45
4% Fe oxide + 3 t/ha OM	82.42±5.54	160.65±16.22
0.5% Fe oxide + 6 t/ha OM	121.41±41.67	170.53±12.77
1% Fe oxide + 6 t/ha OM	121.27±18.19	161.42±14.20
2% Fe oxide + 6 t/ha OM	94.25±25.29	159.32±47.55
4% Fe oxide + 6 t/ha OM	107.33±21.65	158.22±54.75
0.5% Fe oxide + 12 t/ha OM	113.84±37.44	200.19±28.79
1% Fe oxide + 12 t/ha OM	94.68±10.49	152.76±38.65
2% Fe oxide + 12 t/ha OM	115.37±15.34	255.30±67.74
4% Fe oxide + 12 t/ha OM	136.65±19.61	172.33±36.16

Table 5- 8: Three-way analysis of variance results of the effects of Fe oxide and Organic matter and time on the masses of total organic carbon in (S+C) fractions (mg) obtained from >250 µm fraction (data did not pass the normality test P<0.05, passed equal variance test P=0.134).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	26967.676	6741.919	6.545	<0.001
OM	4	46582.794	11645.699	11.305	<0.001
Time	1	94798.057	94798.057	92.024	<0.001
Fe + OM	16	46180.107	2886.257	2.802	<0.001
Fe + time	4	16384.979	4096.245	3.976	0.005
OM + time	4	7295.794	1823.949	1.771	0.141
Fe + OM + time	16	33114.901	2069.681	2.009	0.019

5.3.5.3. Changes in mass of Sand and stable aggregates (S+A) (obtained from 63-250 µm fraction)

Masses of sand and stable aggregate (S+A) fractions obtained from 63-250 µm fractions are shown in Table 5-9. Masses of S+A fractions did not differ significantly due to any of the treatments (Table 5-10)

Table 5- 9: Mean masses of sand and stable aggregate (S+A) fractions obtained from 63-250 µm fractions before and after the experiment (n=3, ± standard deviation).

Treatments	Mass of S+A fraction (g) before 8 weeks	Mass of S+A fraction (g) after 8 weeks
Control	9.75±1.02	9.41±1.66
0.5 % Fe oxide	9.72±1.09	9.70±1.38
1% Fe oxide	9.53±2.54	8.63±2.26
2% Fe oxide	8.85±1.81	10.64±1.01
4% Fe oxide	9.56±1.42	9.85±1.39
1.5 t/ha OM	9.28±3.10	9.15±1.21
3 t/ha OM	8.83±2.56	11.27±2.03
6 t/ha OM	8.71±1.08	10.70±1.15
12 t/ha OM	9.0±0.81	9.52±1.86
0.5% Fe oxide +1.5 t/ha OM	9.56±1.51	9.27±1.11
1% Fe oxide + 1.5 t/ha OM	9.86±1.01	10.70±2.06
2% Fe oxide + 1.5 t/ha OM	9.19±2.04	9.15±2.09
4% Fe oxide + 1.5 t/ha OM	9.99±2.48	10.12±0.24
0.5% Fe oxide + 3 t/ha OM	10.55±2.10	8.31±1.98
1% Fe oxide + 3 t/ha OM	8.45±0.88	8.73±1.88
2% Fe oxide + 3 t/ha OM	8.36±1.15	9.60±0.84
4% Fe oxide + 3 t/ha OM	9.78±0.81	9.53±1.34
0.5% Fe oxide + 6 t/ha OM	7.94±1.98	12.18±1.11
1% Fe oxide + 6 t/ha OM	8.86±1.89	9.48±1.42
2% Fe oxide + 6 t/ha OM	10.29±3.10	9.59±1.03
4% Fe oxide + 6 t/ha OM	8.62±1.34	10.50±1.42
0.5% Fe oxide + 12 t/ha OM	10.02±1.92	8.39±1.32
1% Fe oxide + 12 t/ha OM	9.49±2.25	10.35±0.66
2% Fe oxide + 12 t/ha OM	8.58±1.53	8.88±0.58
4% Fe oxide + 12 t/ha OM	8.85±0.92	9.69±0.75

Table 5- 10: Three-way analysis of variance results of the effects of Fe oxide and Organic matter and time on the masses of sand and stable aggregate (S+A) fractions obtained from 63-250 µm fractions (data passed the normality test P=0.085, and equal variance test, P= 0.996).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	2.221	0.555	0.203	0.936
OM	4	3.914	0.979	0.358	0.838
Time	1	8.262	8.262	3.022	0.085
Fe oxide + OM	16	20.871	1.304	0.477	0.953
Fe oxide + Time	4	3.164	0.791	0.289	0.884
OM + Time	4	12.244	3.061	1.120	0.352
Fe oxide + OM + Time	16	48.978	3.061	1.120	0.348

5.3.5.4. Changes in masses of particulate organic matter (POM) fractions (obtained from fraction >250 µm)

Masses of POM obtained from the macro-aggregates (> 250 µm) are presented in Table 5-11. Masses of POM did not differ significantly within any of the treatments except experimental time (Table 5-12.). The masses of POM fractions were higher by the end of the experiment compared to the beginning.

Table 5- 11: Mean masses of particulate organic matter (POM) (obtained from fraction >250 µm) fractions before and after the experiment (n=3, ± standard deviation).

Treatments	Masses of POM (g) fractions (>250 µm) before 8 weeks	Masses of POM (g) fractions (>250 µm) after 8 weeks
Control	0.13±0.21	0.28±0.12
0.5 % Fe oxide	0.24±0.19	0.22±0.17
1% Fe oxide	0.11±0.011	0.22±0.05
2% Fe oxide	0.30±0.34	0.15±0.05
4% Fe oxide	0.08±0.007	0.27±0.19
1.5 t/ha OM	0.20±0.09	0.37±0.26
3 t/ha OM	0.09±0.04	0.36±0.21
6 t/ha OM	0.22±0.09	0.38±0.28
12 t/ha OM	0.31±0.22	0.43±0.35
0.5 % Fe oxide + 1.5 t/ha OM	0.09±0.02	0.29±0.18
1% Fe oxide + 1.5 t/ha OM	0.18±0.051	0.36±0.05
2% Fe oxide + 1.5 t/ha OM	0.10±0.02	0.40±0.17
4% Fe oxide + 1.5 t/ha OM	0.14±0.01	0.38±0.36
0.5% Fe oxide + 3 t/ha OM	0.19±0.12	0.12±0.02
1% Fe oxide + 3 t/ha OM	0.33±0.41	0.38±0.27
2% Fe oxide + 3 t/ha OM	0.10±0.02	0.36±0.08
4% Fe oxide + 3 t/ha OM	0.14±0.01	0.41±0.05
0.5% Fe oxide + 6 t/ha OM	0.17±0.08	0.27±0.11
1% Fe oxide + 6 t/ha OM	0.17±0.08	0.12±0.07
2% Fe oxide + 6 t/ha OM	0.11±0.08	0.43±0.08
4% Fe oxide + 6 t/ha OM	0.13±0.06	0.23±0.21
0.5% Fe oxide + 12 t/ha OM	0.43±0.14	0.42±0.35
1% Fe oxide + 12 t/ha OM	0.23±0.14	0.31±0.18
2% Fe oxide + 12 t/ha OM	0.31±0.15	0.31±0.25
4% Fe oxide + 12 t/ha OM	0.27±0.05	0.20±0.05

Table 5- 12: Three-way analysis of variance results of the effects of Fe oxide, organic matter, and time on the masses of particulate organic matter (POM) obtained from fraction >250 µm fractions (data were normalised by using log(10) P= 0.136, passed the Equal Variance Test P= 0.364).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	0.153	0.0382	0.414	0.798
OM	4	0.904	0.226	2.445	0.051
Time	1	1.213	1.213	13.119	<0.001
Fe oxide + OM	16	0.728	0.0455	0.492	0.946
Fe oxide + Time	4	0.318	0.0794	0.859	0.491
OM + Time	4	0.590	0.147	1.595	0.182
Fe oxide + OM + Time	16	1.564	0.0977	1.058	0.405

5.3.5.5. Changes in masses of POM fractions (obtained from 63-250 µm fraction)

Mean masses of POM obtained from 63-250 µm fractions are shown in Table 5-13. The amount of POM was not affected by any of the treatments except experimental time (Table 5-14.). At the end of the experiment, the masses of POM obtained from 63-250 µm fractions significantly decreased.

Table 5- 13: Mean masses of particulate organic matter (obtained from fraction 63-250 µm) fractions before and after the experiment (n=3, ± standard deviation).

Treatments	Mass of POM (g) in 63-250µm before 8 weeks	Mass of POM (g) in 63-250µm after 8 weeks
Control	0.12±0.01	0.20±0.09
0.5 % Fe oxide	0.13±0.01	0.12±0.05
1% Fe oxide	0.15±0.04	0.13±0.05
2% Fe oxide	0.34±0.03	0.13±0.03
4% Fe oxide	0.24±0.12	0.16±0.05
1.5 t/ha OM	0.18±0.13	0.11±0.12
3 t/ha OM	0.33±0.18	0.12±0.04
6 t/ha OM	0.20±0.09	0.10±0.07
12 t/ha OM	0.22±0.01	0.19±0.08
0.5 % Fe oxide + 1.5 t/ha OM	0.19±0.08	0.09±0.02
1% Fe oxide + 1.5 t/ha OM	0.19±0.12	0.18±0.08
2% Fe oxide + 1.5 t/ha OM	0.25±0.09	0.07±0.07
4% Fe oxide + 1.5 t/ha OM	0.16±0.02	0.19±0.07
0.5% Fe oxide + 3 t/ha OM	0.22±0.03	0.23±0.03
1% Fe oxide + 3 t/ha OM	0.12±0.05	0.10±0.04
2% Fe oxide + 3 t/ha OM	0.17±0.06	0.14±0.10
4% Fe oxide + 3 t/ha OM	0.20±0.08	0.11±0.08
0.5% Fe oxide + 6 t/ha OM	0.16±0.06	0.18±0.07
1% Fe oxide + 6 t/ha OM	0.13±0.01	0.13±0.02
2% Fe oxide + 6 t/ha OM	0.19±0.1	0.05±0.01
4% Fe oxide + 6 t/ha OM	0.22±0.05	0.27±0.12
0.5% Fe oxide + 12 t/ha OM	0.24±0.03	0.16±0.12
1% Fe oxide + 12 t/ha OM	0.26±0.20	0.21±0.10
2% Fe oxide + 12 t/ha OM	0.19±0.04	0.19±0.04
4% Fe oxide + 12 t/ha OM	0.12±0.10	0.19±0.06

Table 5- 14: Three-way analysis of variance results of the effects of Fe oxide, organic matter and time on the masses of POM fractions obtained from 63-250 µm fraction (data passed the normality test P =0.757, and Equal Variance Test P= 0.326).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	0.00998	0.00250	0.355	0.840
OM	4	0.0269	0.00672	0.955	0.436
Time	1	0.0843	0.0843	11.989	<0.001
Fe oxide + OM	16	0.190	0.0119	1.692	0.060
Fe oxide + time	4	0.0495	0.0124	1.760	0.143
OM + time	4	0.0131	0.00328	0.466	0.761
Fe oxide + OM + time	16	0.166	0.0104	1.479	0.123

5.3.5.6. Changes in masses of silt + clay (S+C) fractions (0.45-63 µm)

Masses of silt + clay fractions (0.45-63 µm) are presented in Table 5-15. The masses of silt + clay fractions did not change significantly within any of the treatments except experimental time. After 8 weeks, the masses of S+C were significantly lower compared to before regardless of what level of treatment was given (Table 5-16.).

Table 5- 15: Mean masses of silt + clay (S+C) fractions (g) before and after the experiment (n=3, ± standard deviation).

Treatments	Mass of S+C (g) fraction before 8 weeks	Mass of S+C fraction (g) after 8 weeks
Control	8.17±1.26	6.88±0.69
0.5 % Fe oxide	8.39±0.90	5.94±0.99
1% Fe oxide	8.17±1.75	6.84±2.14
2% Fe oxide	9.98±1.80	7.00±2.37
4% Fe oxide	9.66±0.15	6.35±1.12
1.5 t/ha OM	8.46±1.11	7.50±1.60
3 t/ha OM	9.14±1.12	6.21±0.63
6 t/ha OM	6.81±1.02	6.80±1.58
12 t/ha OM	7.83±1.91	5.68±1.86
0.5 % Fe oxide + 1.5 t/ha OM	7.39±0.26	7.50±0.70
1% Fe oxide + 1.5 t/ha OM	7.30±0.69	5.78±0.51
2% Fe oxide + 1.5 t/ha OM	7.48±1.87	6.41±1.55
4% Fe oxide + 1.5 t/ha OM	6.54±1.67	7.35±1.47
0.5% Fe oxide + 3 t/ha OM	8.21±1.21	6.90±0.06
1% Fe oxide + 3 t/ha OM	9.05±0.92	6.41±0.50
2% Fe oxide + 3 t/ha OM	9.34±0.81	6.59±0.84
4% Fe oxide + 3 t/ha OM	8.54±0.53	6.50±2.92
0.5% Fe oxide + 6 t/ha OM	7.36±0.54	7.22±0.65
1% Fe oxide + 6 t/ha OM	8.26±0.92	6.18±1.25
2% Fe oxide + 6 t/ha OM	8.63±2.04	6.56±0.88
4% Fe oxide + 6 t/ha OM	8.90±0.70	6.89±2.32
0.5% Fe oxide + 12 t/ha OM	7.66±1.11	7.40±0.71
1% Fe oxide + 12 t/ha OM	8.64±0.53	7.90±1.61
2% Fe oxide + 12 t/ha OM	7.18±1.03	6.19±1.28
4% Fe oxide + 12 t/ha OM	8.08±2.12	6.93±0.88

Table 5- 16: Three-way analysis of variance results of the effects of Fe oxide, organic matter, and time on the masses of silt + clay (S+C) fractions (0.45-63 µm) (data passed the normality test P =0.771, and equal variance test P= 0.877).

Source of Variation	DF	SS	MS	F	p
Fe oxide	4	0.868	0.217	0.121	0.975
OM	4	9.621	2.405	1.340	0.260
Time	1	81.019	81.019	45.155	<0.001
Fe oxide + OM	16	25.295	1.581	0.881	0.592
Fe oxide + Time	4	7.885	1.971	1.099	0.362
OM + time	4	17.494	4.374	2.438	0.052
Fe oxide + OM + Time	16	15.294	0.956	0.533	0.924

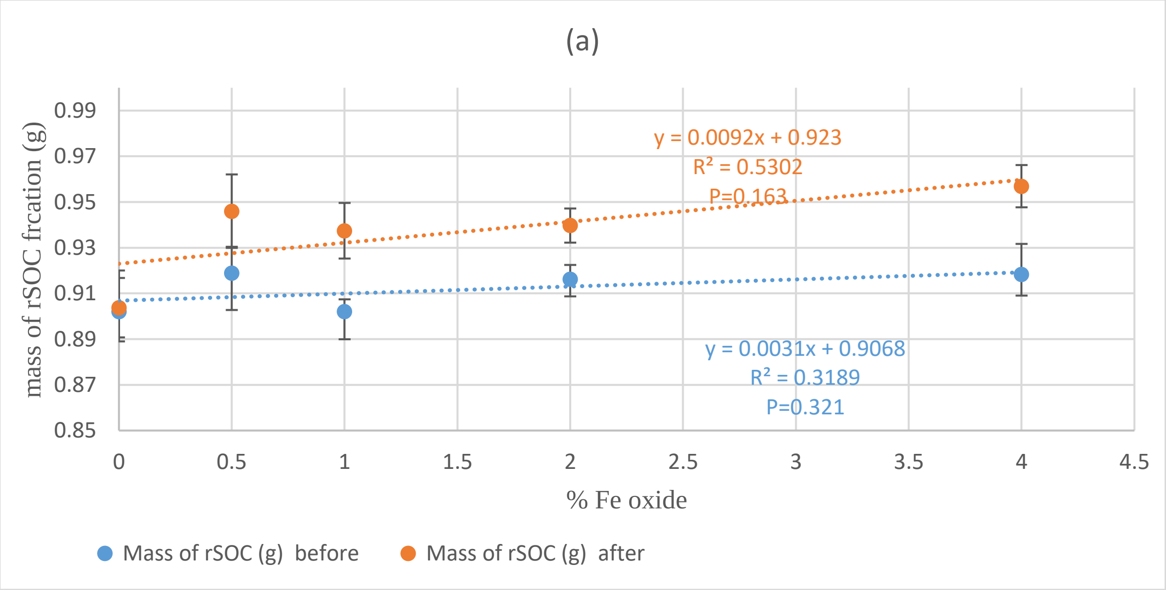
5.3.5.7. Masses of Chemically resistant soil organic carbon (rSOC) fractions (obtained from oxidation of silt + clay fraction)

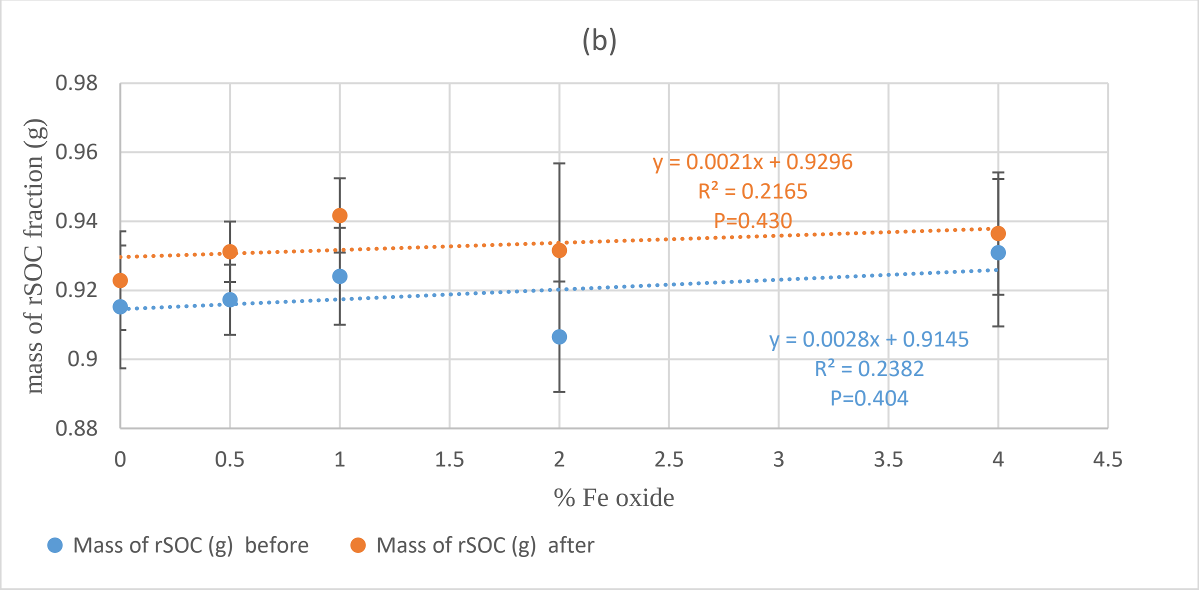
Changes in the masses of chemically resistant soil organic carbon (rSOC) fractions over the incubation period are shown in Figure 5-7. The masses of rSOC fractions significantly increased over the experimental time (P<0.001). There was a significant interaction of Fe oxide and time on the masses of rSOC fraction (Table 5-17.). However, the three-way interaction of Fe oxide +OM +time did not significantly affect the mass.

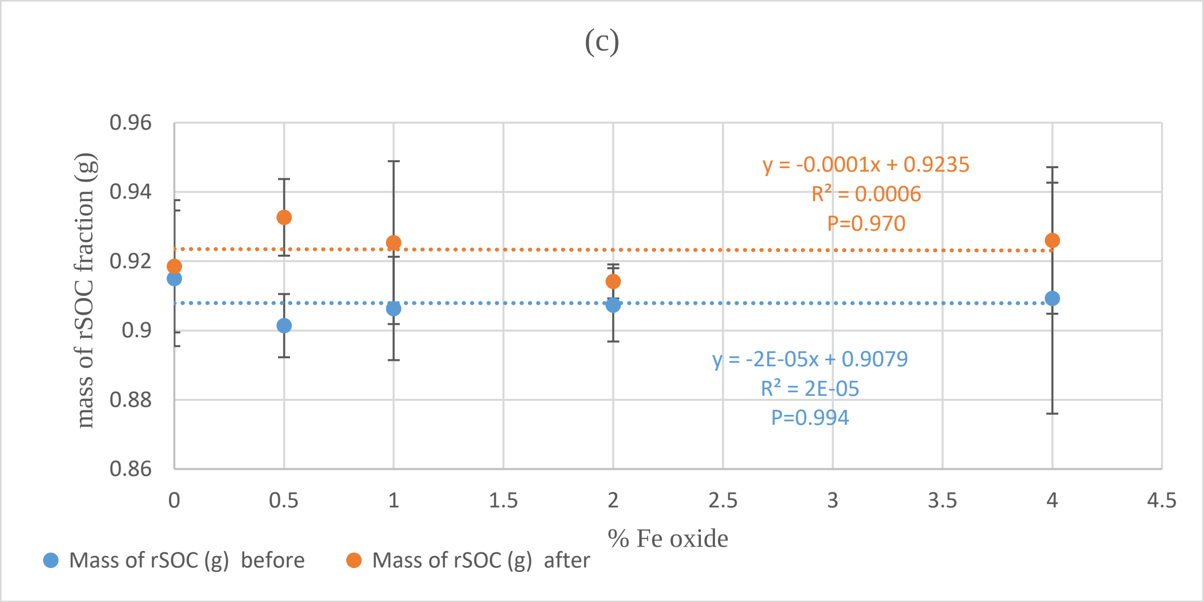
Pairwise multiple comparisons showed that at the beginning of the experiment there was no significant difference in mass due to Fe oxide treatment. After 8 weeks of the experimental period the mass of rSOC was significantly higher (P< 0.05) in all the different levels of Fe oxide treatment (except 0 % Fe oxide) compared to before. Comparison between the Fe oxide treatments at the end of the experiment showed that the rSOC fraction in the 0.5% and 2% Fe oxide treatments had significantly higher mass (P= 0.001, 0.019 respectively) compared to the control condition.

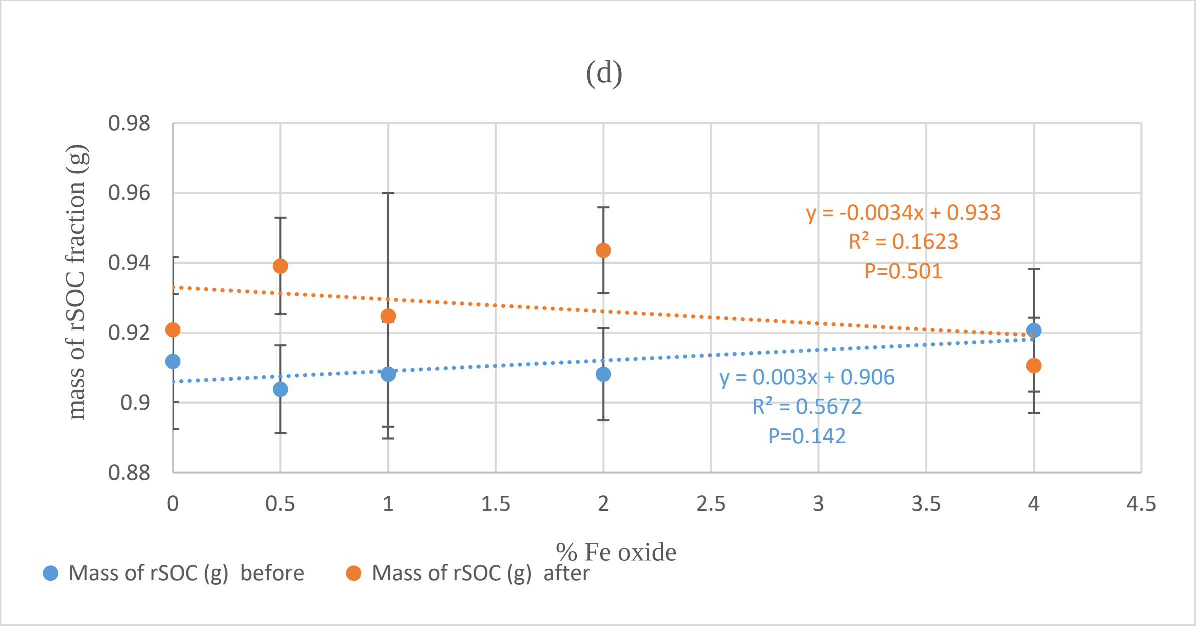
Table 5- 17: Three-way analysis of variance results of the effects of Fe oxide, organic matter, and time on the masses of chemically resistant soil organic carbon (rSOC) fractions (obtained from oxidation of silt + clay fraction) (data passed the normality test P = 0.557, and the equal variance test P= 0.461).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	0.00246	0.000615	2.305	0.063
OM	4	0.00234	0.000585	2.192	0.075
Time	1	0.0128	0.0128	47.814	<0.001
Fe oxide + OM	16	0.00528	0.000330	1.237	0.254
Fe oxide + Time	4	0.00335	0.000837	3.137	0.018
OM + Time	4	0.000607	0.000152	0.569	0.686
Fe oxide + OM + Time	16	0.00338	0.000211	0.792	0.691




(b)
mass of rSOC fraction (g)
0.98
0.96
0.94
0.92
0.9
0.88
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
y = 0.0021x + 0.9296
R² = 0.2165
P=0.430
y = 0.0028x + 0.9145
R² = 0.2382
P=0.404
● Mass of rSOC (g) before
● Mass of rSOC (g) after


(c)
mass of rSOC fraction (g)
0.96
0.94
0.92
0.9
0.88
0.86
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
y = -0.0001x + 0.9235
R² = 0.0006
P=0.970
y = -2E-05x + 0.9079
R² = 2E-05
P=0.994
● Mass of rSOC (g) before
● Mass of rSOC (g) after


(d)
mass of rSOC fraction (g)
0.98
0.96
0.94
0.92
0.9
0.88
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
y = -0.0034x + 0.933
R² = 0.1623
P=0.501
y = 0.003x + 0.906
R² = 0.5672
P=0.142
● Mass of rSOC (g) before
● Mass of rSOC (g) after

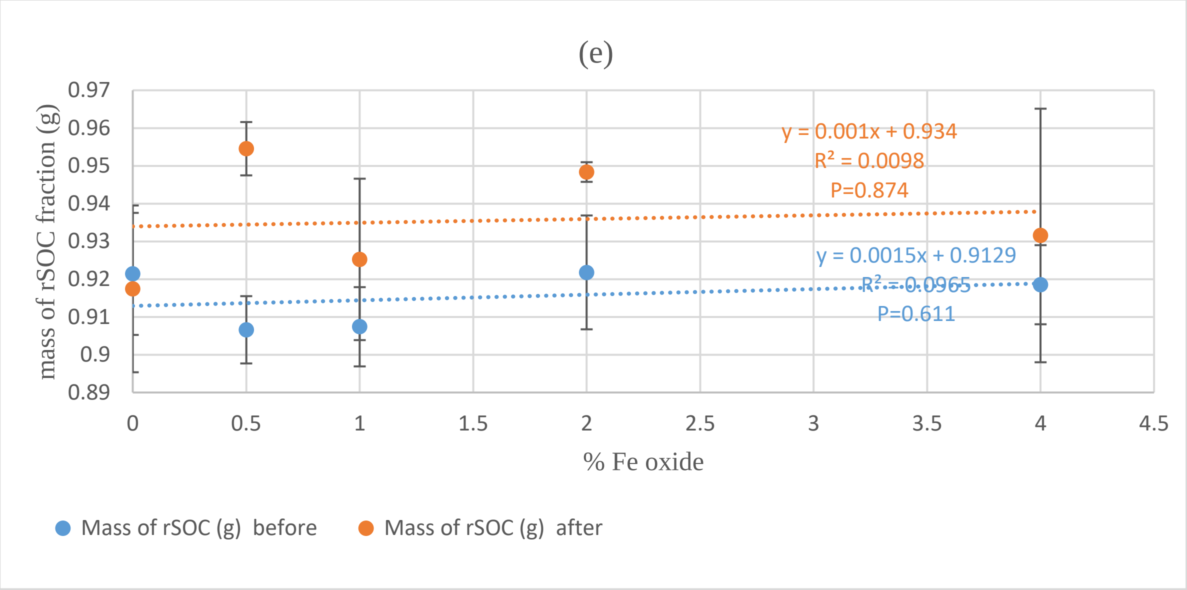


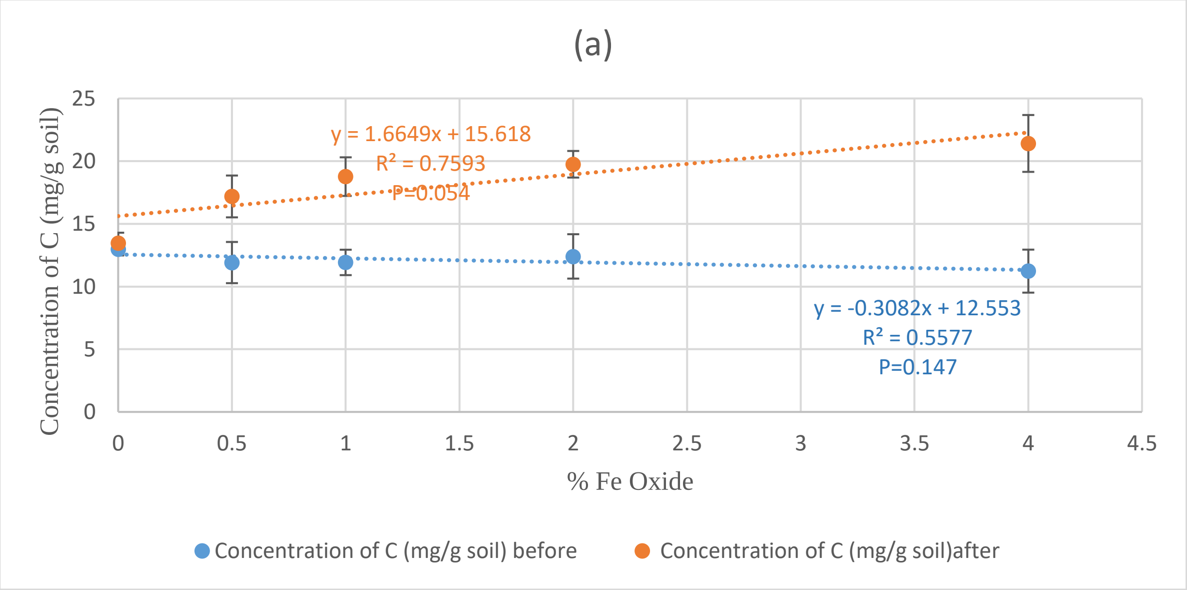
Figure 5- 7: Changes in absolute mass of resistant soil organic carbon (rSOC) fraction over the experimental time due to different levels of Fe oxide (%) treatments at (a) 0 t/ha OM (b) 1.5 t/ha OM (c) 3 t/ha OM (d) 6t/ha OM and (e) 12 t/ha OM (n=3, error bars indicate standard deviation).

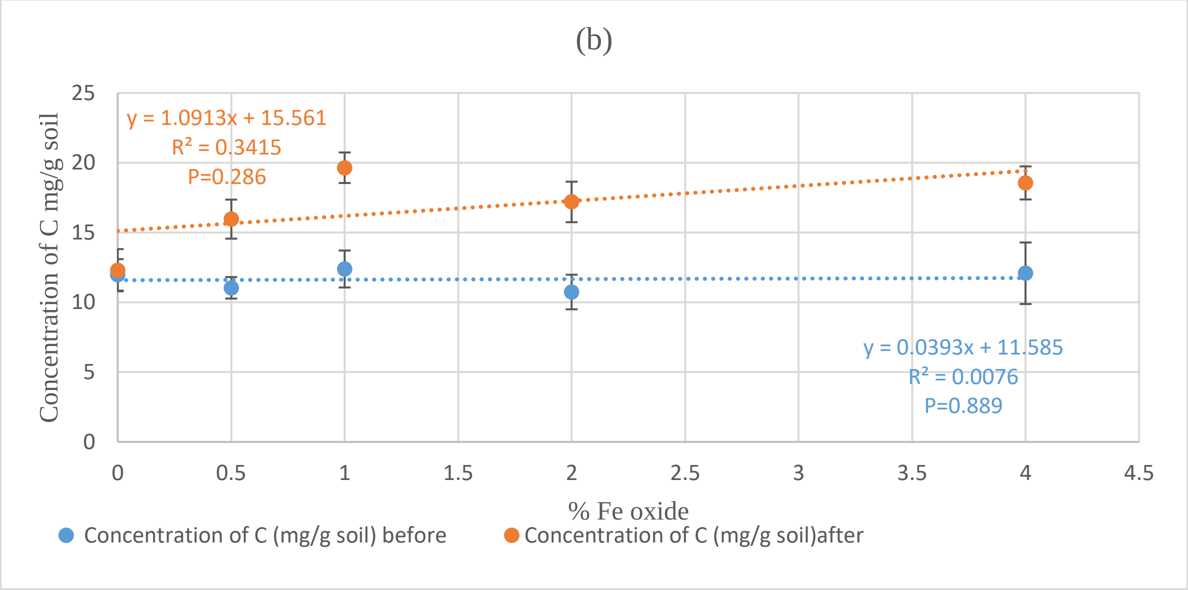
5.3.5.8. Concentration of C (mg/g soil) in rSOC fractions

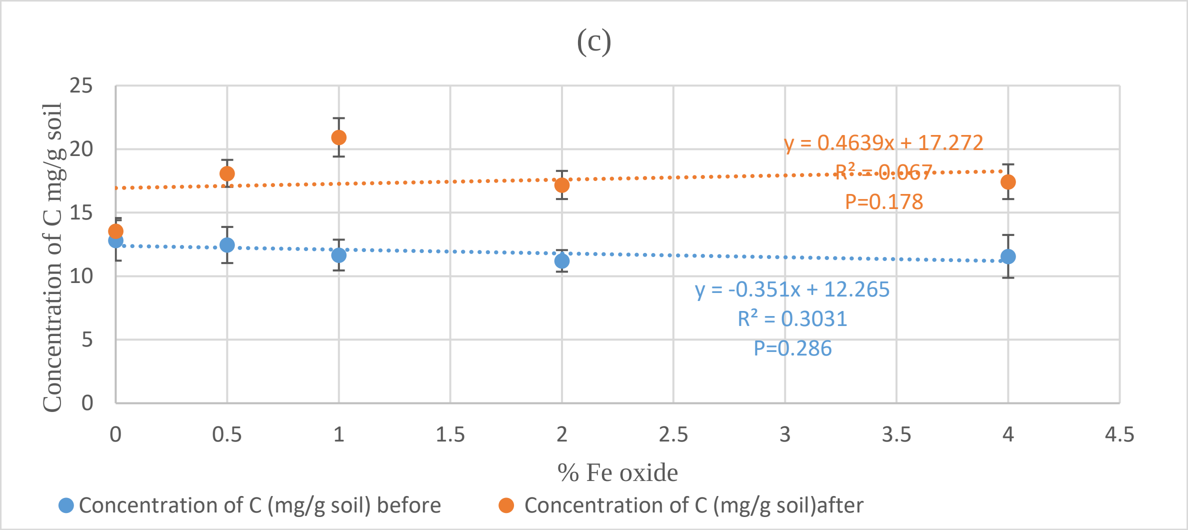
The changes in concentration of organic carbon (mg/g soil) in rSOC fractions over the incubation period are shown in Figure 5-8. The effect of Fe oxide, OM, and Time was significant ($P < 0.05$) on the concentration of organic carbon in rSOC fraction. The difference in the concentration of organic carbon due to the interactive effect of Fe oxide + time was significant ($P < 0.001$) (Table 5-18.). There was no significant interaction between Fe oxide + OM (P=0.078), OM + Time (P= 0.631), and Fe oxide + OM + Time (P= 0.544).

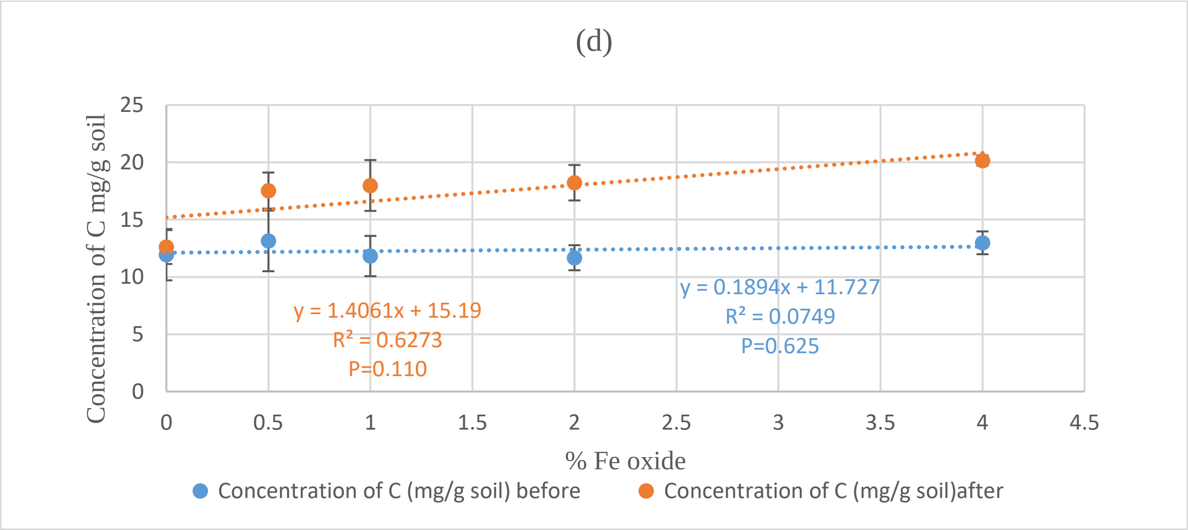
Table 5- 18: Three-way analysis of variance results of the effects of Fe oxide, organic matter and time on the concentration of organic C (mg/g soil) in rSOC fractions (data not pass the normality test and normalised by simple transformation with log(10) P = 0.092, passed the equal variance test P= 0.987).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	0.126	0.0316	15.532	<0.001
OM	4	0.0364	0.00910	4.472	0.002
Time	1	0.892	0.892	438.142	<0.001
Fe oxide + OM	16	0.0526	0.00329	1.616	0.078
Fe oxide + Time	4	0.152	0.0379	18.635	<0.001
OM + Time	4	0.00527	0.00132	0.647	0.631
Fe oxide + OM + Time	16	0.0301	0.00188	0.925	0.544




(b)
Concentration of C mg/g soil
% Fe oxide
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
y = 1.0913x + 15.561
R² = 0.3415
P=0.286
y = 0.0393x + 11.585
R² = 0.0076
P=0.889
● Concentration of C (mg/g soil) before
● Concentration of C (mg/g soil)after


(c)
Concentration of C mg/g soil
% Fe oxide
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
y = 0.4639x + 17.272
R² = 0.067
P=0.178
y = -0.351x + 12.265
R² = 0.3031
P=0.286
● Concentration of C (mg/g soil) before
● Concentration of C (mg/g soil)after


(d)
Concentration of C mg/g soil
% Fe oxide
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
y = 1.4061x + 15.19
R² = 0.6273
P=0.110
y = 0.1894x + 11.727
R² = 0.0749
P=0.625
● Concentration of C (mg/g soil) before
● Concentration of C (mg/g soil)after

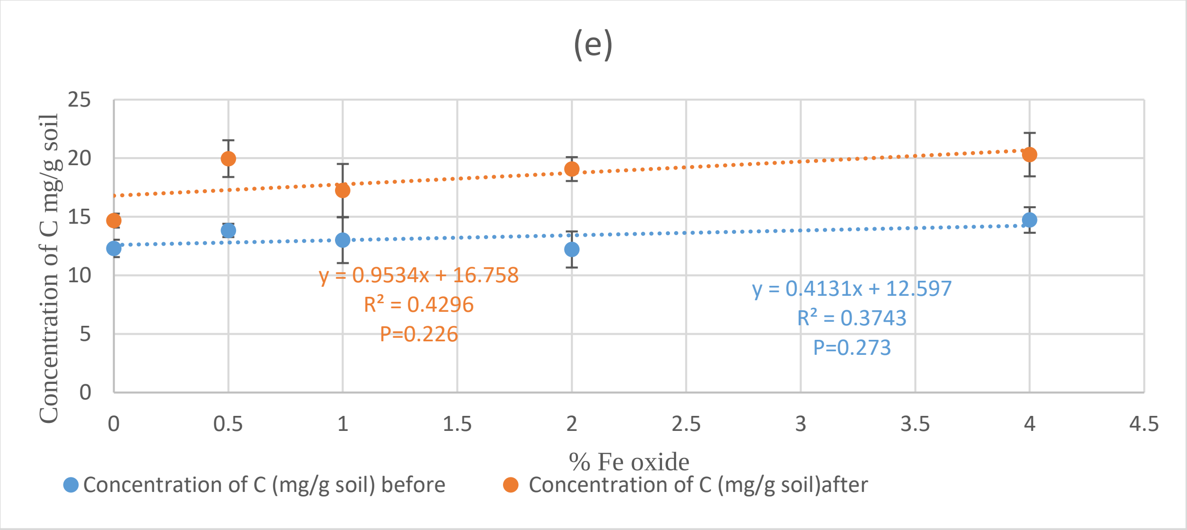


Figure 5- 8: Changes in the concentration of organic carbon in the resistant soil organic carbon fraction (rSOC) over the experimental time due to different levels of Fe oxide (%) treatments at (a) 0 t/ha OM (b) 1.5 t/ha OM (c) 3 t/ha OM (d) 6t/ha OM and (e) 12 t/ha OM (n=3, error bars indicate standard deviation).

Pairwise multiple comparisons found that the concentration of carbon in the rSOC fraction was significantly higher at the end of the experiment compared to the beginning ($P< 0.001$) regardless of any treatments.

After 8 weeks, Fe oxide treatment showed significantly higher ($P< 0.001$) concentration of organic carbon in all the different levels, (except 0 % Fe oxide treatment) than that of before. 4% Fe oxide treatment had the highest concentration of organic carbon in rSOC fraction by the end of the experiment. There was no significant difference in organic carbon content within different levels of Fe oxide treatment before the experiment.

We calculated the total amount of organic carbon (mg) in each fraction [mass of rSOC fraction (g) multiplied by the organic carbon concentration (mg/g soil)] and observed if there is any difference in the effects of our treatments on the carbon content in rSOC fractions (Table 5-19). A three-way analysis of variance (ANOVA) test found that increasing Fe oxide amendment caused a significant increase in the amount of total organic carbon in rSOC fractions which was the same observation as we found before for the concentration of organic carbon in rSOC fractions. We found there was a significant interaction between Fe oxide and OM (Table 5-20).

All pairwise multiple comparisons showed that with a particular level of Fe oxide treatment adding OM did not cause any significant difference in the amount of total organic carbon in rSOC fractions. However, we observed many different combinations of Fe oxides +OM, where adding Fe oxide treatments with a particular level of OM significantly increased the amount of total organic carbon in rSOC fractions. 1.5, 3, and 6 t/ha OM with 1% Fe oxide treatment had significantly higher amount of total organic carbon than that of the individual effect of OM. Similarly, the amount of total organic carbon in rSOC fractions was significantly higher at 1.5 t/ha OM and 12 t/ha OM with 4% Fe oxide than that in 1.5 t/ha or 12 t/ha OM with no Fe oxide treatment. This result indicates that there was no significant increase in rSOC with OM additions.

Overall Fe oxide treatments caused an increase in organic carbon concentration in rSOC fractions. The effect of OM and their interaction with Fe oxide did not significantly increase the carbon concentration in rSOC fractions over the incubation period.

Table 5- 19: Mean masses of total organic carbon (mg) in rSOC fractions before and after the experiment (n=3, ± standard deviation).

Treatments	Mean masses of total organic carbon (mg) in rSOC fractions before the experiment	Mean masses of total organic carbon (mg) in rSOC fractions after the experiment
Control	11.71±0.39	12.15±0.5
0.5 % Fe oxide	10.95±1.05	16.24±1.4
1% Fe oxide	10.75±0.91	17.59±1.6
2% Fe oxide	11.36±1.67	18.56±1.04
4% Fe oxide	10.32±1.68	20.49±2.25
1.5 t/ha OM	10.95±1.07	11.35±1.38
3 t/ha OM	11.73±1.62	12.44±0.97
6 t/ha OM	10.91±2.28	11.62±1.49
12 t/ha OM	11.33±0.57	13.47±0.79
0.5 % Fe oxide + 1.5 t/ha OM	10.12±0.62	14.85±1.17
1% Fe oxide + 1.5 t/ha OM	11.46±1.39	18.49±1.01
2% Fe oxide + 1.5 t/ha OM	9.74±1.18	16.03±1.72
4% Fe oxide + 1.5 t/ha OM	11.25±2.11	17.36±0.84
0.5% Fe oxide + 3 t/ha OM	11.22±1.29	16.86±0.79
1% Fe oxide + 3 t/ha OM	10.58±1.24	19.38±1.90
2% Fe oxide + 3 t/ha OM	10.15±0.62	15.70±1.09
4% Fe oxide + 3 t/ha OM	10.48±1.30	16.14±1.32
0.5% Fe oxide + 6 t/ha OM	11.86±2.30	16.47±1.67
1% Fe oxide + 6 t/ha OM	10.75±1.77	16.67±2.56
2% Fe oxide + 6 t/ha OM	10.60±1.10	17.19±1.49
4% Fe oxide + 6 t/ha OM	11.95±1.01	18.35±0.57
0.5% Fe oxide + 12 t/ha OM	12.54±0.59	19.06±1.57
1% Fe oxide + 12 t/ha OM	11.78±1.62	15.98±2.45
2% Fe oxide + 12 t/ha OM	11.26±1.51	18.09±1.01
4% Fe oxide + 12 t/ha OM	13.53±1.15	18.90±1.69

Table 5- 20: Three-way analysis of variance results of the effects of Fe oxide, organic matter and time on the masses of total organic carbon (mg) in rSOC fractions (data did not pass the normality test P <0.05, passed the equal Variance Test P= 0.999).

Source of Variation	DF	SS	MS	F	P
Fe oxides	4	169.360	42.340	20.338	<0.001
OM	4	36.539	9.135	4.388	0.003
Time	1	1016.457	1016.457	488.243	<0.001
Fe oxide + OM	16	65.156	4.072	1.956	0.024
Fe oxide +time	4	184.520	46.130	22.158	<0.001
OM + time	4	6.557	1.639	0.787	0.536
Fe oxide +OM + time	16	42.433	2.652	1.274	0.229

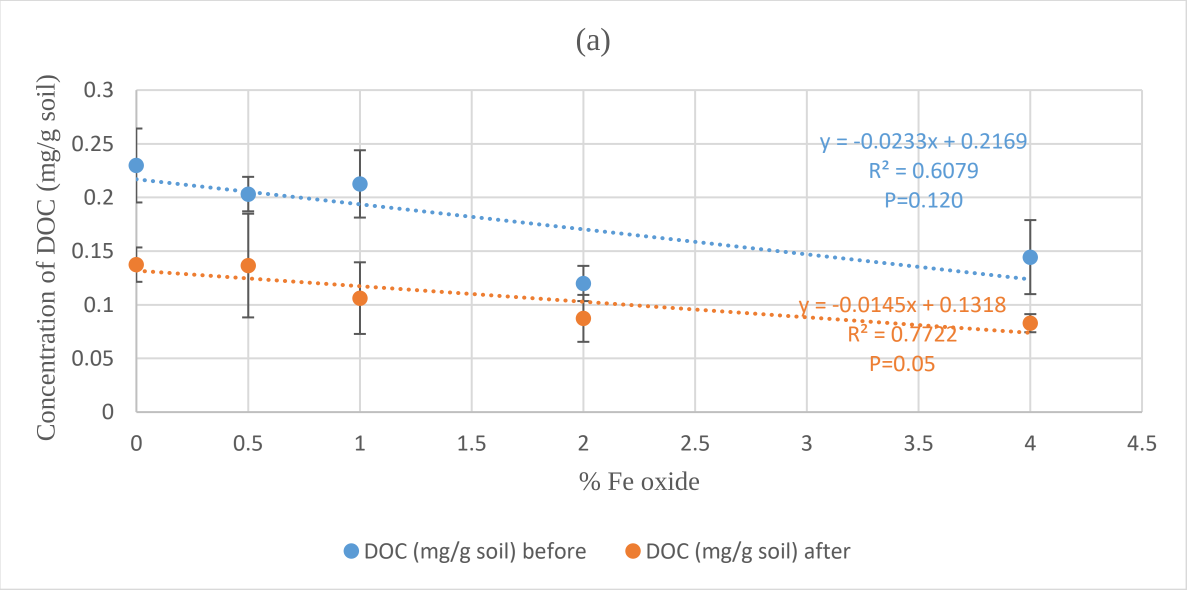
5.3.5.9. Dissolved organic carbon (<0.45 µm)

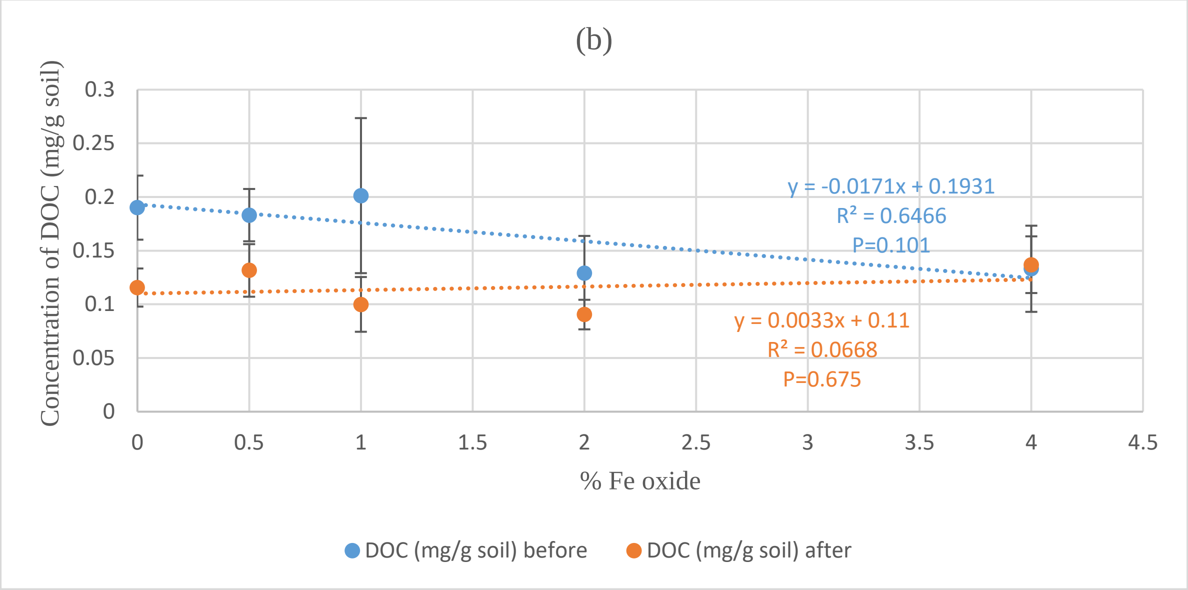
Changes in the concentration of dissolved organic carbon over the experimental period are shown in Figure 5-9. Fe oxide treatment and the experimental time significantly affected (P< 0.001) the concentration of dissolved organic carbon in soil. There is a significant interaction between Fe oxide and time (Table 5-21.). The interaction of Fe oxide + OM, OM + time, or Fe oxide + OM +time did not significantly affect the concentration of dissolved organic carbon (Table 5-21.).

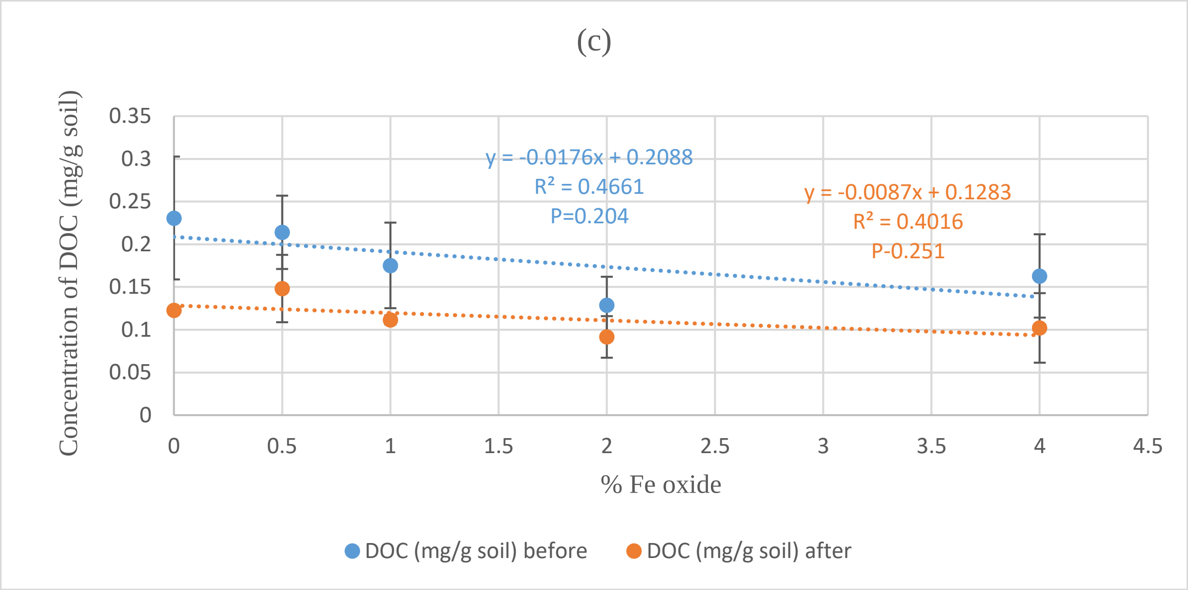
Pairwise multiple comparisons found that by the end of 8 weeks, the concentration of dissolved organic carbon was significantly lower within all Fe oxide treatments than that of before 8 weeks (Figure 5-9). At the beginning of the experiment the concentration of dissolved organic carbon was significantly lower (P<0.001) at 4% and 2% Fe oxide treatment compared to control and 0.5% Fe oxide treatment. In addition, significantly lower concentrations of DOC was observed in 4% and 2% Fe oxide treatments than that of 1% Fe oxide treatment (P<0.05). By the end of the experimental period, DOC was significantly lower in 1, 2, and 4 % Fe oxide treatments compared to 0.5 % Fe oxide treatment (P<0.05). This result suggests that the addition of higher amounts of Fe oxide had a greater effect in reducing the concentration of DOC in soil.

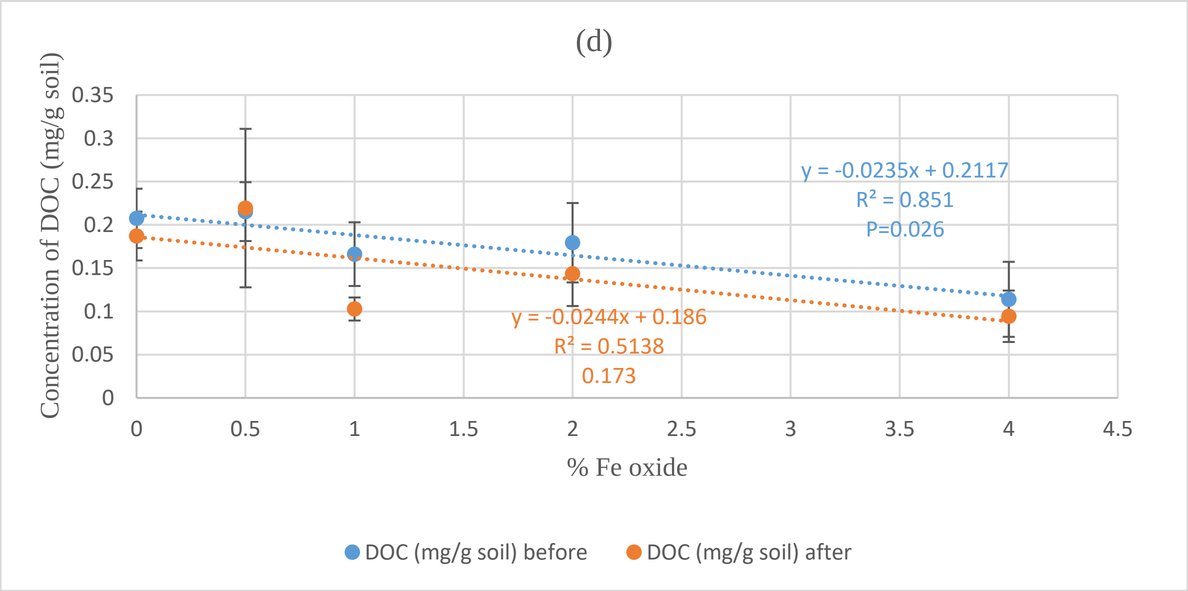
Table 5- 21: Three-way analysis of variance results of the effects of Fe oxide, organic matter and time on the concentration of dissolved organic carbon (data passed normality test P = 0.97 and the equal Variance Test P= 0.897).

Source of Variation	DF	SS	MS	F	P
Fe oxide	4	0.0828	0.0207	15.126	<0.001
OM	4	0.00809	0.00202	1.478	0.215
Time	1	0.122	0.122	88.978	<0.001
Fe oxide + OM	16	0.0343	0.00215	1.568	0.092
Fe oxide + Time	4	0.0136	0.00341	2.491	0.048
OM + Time	4	0.0101	0.00253	1.851	0.125
Fe organic matter + OM + Time	16	0.0117	0.000733	0.536	0.922




(b)
Concentration of DOC (mg/g soil)
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
y = -0.0171x + 0.1931
R² = 0.6466
P=0.101
y = 0.0033x + 0.11
R² = 0.0668
P=0.675
● DOC (mg/g soil) before
● DOC (mg/g soil) after


(c)
Concentration of DOC (mg/g soil)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
y = -0.0176x + 0.2088
R² = 0.4661
P=0.204
y = -0.0087x + 0.1283
R² = 0.4016
P-0.251
● DOC (mg/g soil) before
● DOC (mg/g soil) after


(d)
Concentration of DOC (mg/g soil)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
% Fe oxide
y = -0.0235x + 0.2117
R² = 0.851
P=0.026
y = -0.0244x + 0.186
R² = 0.5138
0.173
● DOC (mg/g soil) before
● DOC (mg/g soil) after

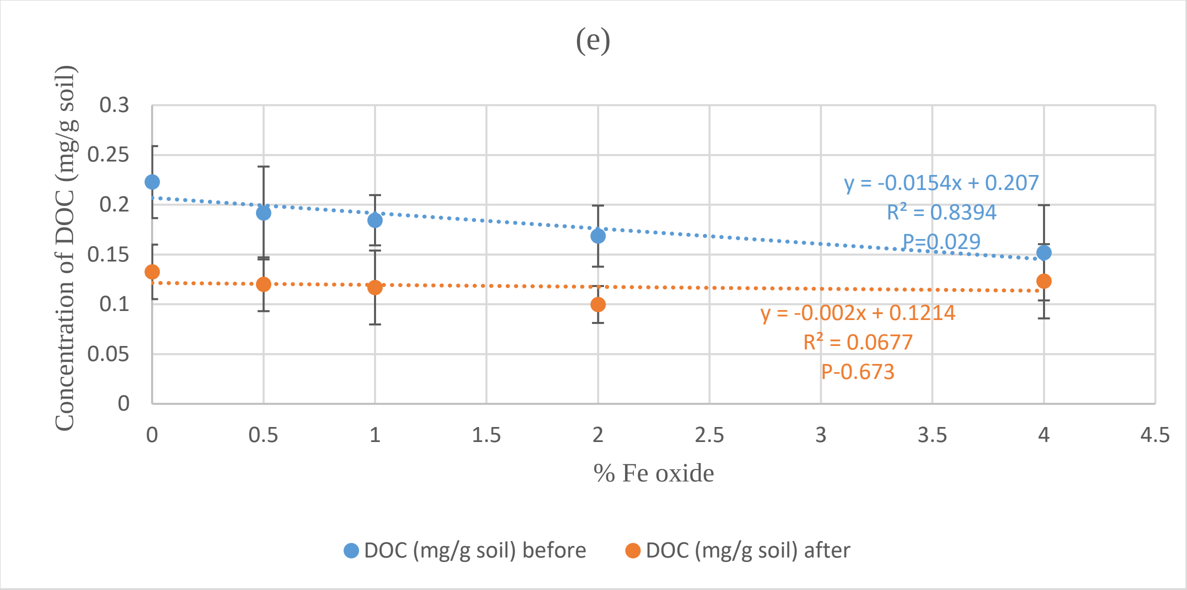


Figure 5- 9: Changes in the concentration of dissolved organic carbon (DOC) over the experimental time due to different levels of Fe oxide (%) treatments at (a) 0 t/ha OM (b) 1.5 t/ha OM (c) 3 t/ha OM (d) 6t/ha OM and (e) 12 t/ha OM (n=3, error bars indicate standard deviation)

5.3.6. Correlations between interrelated soil properties

Based on references from previous studies we decided to determine the correlations between some of the interrelated soil properties given in Table 5-22. Previous studies suggested that soil pH affects the solubility of Fe oxide (discussed in Chapter 2, Section, 2.6.1). There is a strong positive correlation between the amount of poorly crystalline Fe oxide and the amount of water-stable aggregates (Duiker et al., 2003). Several studies suggested the correlation between the Fe oxide content and soil organic carbon content (Torn et al., 1997; Rasmussen et al., 2006; Zhang et al., 2013; Porras et al., 2017).

Table 5- 22: Correlation between interrelated soil properties (datasets obtained after 8 weeks of the experiment were used for all pairs).

Pair of related variables	P value	Rs (correlation coefficient)
Soil pH and the concentration of extractable Fe	<0.0001	-0.563
Absolute mass of S+A fraction and the concentration of organic carbon in S+A fraction (>250µm)	<0.0001	0.458
Absolute mass of S+A fraction and the total mass of organic carbon in S+A fraction (>250µm)	<0.0001	0.824
Concentration of extractable Fe and the absolute mass of S+A fraction (>250µm)	0.00129	0.367
Concentration of extractable Fe and the concentration of organic carbon in S+A fraction (>250µm)	>0.05	0.135
Concentration of extractable Fe and the total amount of organic carbon in S+A fraction (>250µm)	0.0104	0.295
Absolute mass of rSOC fractions and the concentration of carbon in rSOC fraction (0.45-63 µm)	0.000215	0.418
Absolute mass of rSOC fractions and the amount of total carbon in rSOC fraction (0.45-63 µm)	<0.0001	0.988
Concentration of extractable Fe and absolute mass of rSOC fraction (0.45-63 µm)	>0.05	0.132
Concentration of extractable Fe and concentration of carbon in rSOC fraction (0.45-63 µm)	<0.0001	0.495
Concentration of extractable Fe and total amount of organic carbon in rSOC fraction (0.45-63 µm)	<0.0001	0.484
Concentration of extractable Fe and concentration of dissolved organic carbon (<0.45 µm).	<0.0001	-0.433

5.4. Discussion

In this experiment, wheat plants were grown for 8 weeks. We examined the effect of Fe oxide, OM, and several combinations of Fe oxide + OM treatments on the masses of stable aggregate fractions as well as the concentration of stable organic carbon in the aggregate fractions before and after 8 weeks. In addition, the effects of these treatments on reducing the concentration of dissolved organic carbon in soil solution over the 8 weeks period were also determined. We examined how all these above mentioned treatments affected plant growth and soil microbial oxygen consumption rate (soil respiration). Changes in soil pH and the concentration of extractable Fe over the experimental period were also determined.

We found that after 8 weeks the masses of soil macro-aggregate (S+A) fractions (obtained from >250 µm fractions) significantly increased due to Fe oxide treatment. There was a lack of trend in the changes in masses of macro-aggregates in different combinations of Fe oxide +OM treatments. The masses of micro-aggregate (S+A) fractions (obtained from 63-250 µm) did not differ significantly due to any of the treatments. The masses of particulate organic matter (POM) obtained from macro-aggregates significantly increased whereas the masses of POM obtained from micro-aggregates significantly decreased after 8 weeks. None of the treatments had any significant effect on the masses of POM fractions. The masses of silt and clay (S+C) fractions (0.45-63 µm) significantly decreased after 8 weeks regardless of any treatments. Fe oxide treatments caused an increase in the masses of resistant soil organic carbon (rSOC) fractions at the end of the experiment. There was no trend in changes in masses of rSOC fractions in OM treatments or Fe oxide +OM treatments.

The concentration of organic carbon increased in the macro-aggregate (S+A) fractions (obtained from >250 µm fractions) due to Fe oxide treatments. Although regression analysis of our data for the concentration of organic carbon in S+A fractions (obtained from >250 µm fractions) in different combinations of Fe oxide +OM treatments showed no trend, the effect of OM and Fe oxide +OM were significant at the end of the experiment. The addition of more OM showed a higher concentration of organic carbon.

The concentration of organic carbon in rSOC fractions increased at the end of the experiment due to Fe oxide treatments. OM or Fe oxide +OM did not significantly affect the concentration of organic carbon in rSOC fractions.

Both before and after 8 weeks, Fe oxide treatment showed a significant decrease in the concentration of dissolved organic carbon.

The addition of OM caused a significant increase in dry matter biomass of the Wheat plants. The interactive effect of Fe oxide +OM was significant on soil respiration rate. By the end of 8 weeks, soil pH decreased and the concentration of extractable Fe increased.

The key findings of this experiment are further discussed below:

5.4.1. Soil respiration

In this experiment, we watered the wheat plants twice a week in order to maintain optimum soil moisture content and microbial activity. OM (sewage sludge) treatments acted as a source of nutrients for both plants and microbes. The individual effect of Fe oxide and OM treatment did not make any significant difference in the soil microbial O_2 consumption rate. However, microbial activity was higher due to the interactive effect of the highest amount of OM (12 t/ha) and the lowest amount of Fe oxide (0.5%) treatment compared to any other treatments. We assume sewage sludge was a source of labile organic carbon in soil (Figure 5-9 shows OM (sewage sludge) addition causes a higher concentration of DOC in soil compared to that in any other treatments for both before and after the incubation period). Our result agreed with the report that an increase in labile organic carbon or nitrogen fertiliser input produces a higher microbial respiration rate (Cleveland and Townsend 2006, Cleveland et al. 2007). In addition, increased soil pH provides higher microbial activity ((Zimmermann, 2002). Result in Section 5.3.3 for changes in soil pH showed that at the end of the experiment, 12 t/ha OM + 0.5% Fe oxide treatment had higher pH compared to any other Fe oxide treatment within 12 t/ha OM(Figure 5-3)

5.4.2. Plant biomass

Wheat plants biomasses increased with an increase in sewage sludge application and were the highest at the highest rate of sewage sludge treatment (12t/ha). We assume sewage sludge in soil acted as a fertiliser and helped increasing plant biomass by providing essential nutrients. This result agreed with several previous studies where sewage sludge amendment significantly increased crop biomass (Latare et al., 2014; Eid et al., 2018)

5.4.3. Changes in soil pH

In this experiment, we used coal mining drainage ochre obtained from an abandoned mining site in the UK as a source of Fe oxides. Ochre is a ferruginous precipitate from coal mine water, which accumulates during the treatment of mine drainage water. It largely comprises poorly crystalline Fe oxide and generally has a low pH (Sapsford et al., 2015; SEPA, 2008). The application of Fe oxide treatment slightly reduced soil pH compared to the unamended soil.

Furthermore, soil pH reduced in all the treatments at the end of the experimental plant growth period. We assume the reason behind pH decline could be the production of organic acid from microbial decomposition of organic matter. Organic matter was added according to the nitrogen requirement of soil, therefore nitrification (release of H^+ ion due to the conversion of nitrogen fertiliser into nitrate) of organic matter could be a reason of reduced soil pH (Tisdale and Nelson, 1970; Russell, 1988; Wong and Lai, 1996).

5.4.4. Changes in the concentration of extractable Fe

A significant negative correlation exists between soil pH and the concentration of extractable Fe (Table 5-22.). Furthermore, the concentration of extractable Fe (crystalline plus non-crystalline) was higher at the end of the experiment regardless of any treatment. We assume that the reason behind the increasing concentration of extractable Fe at the end of 8 weeks could be the decrease in soil pH by the end of the experiment. Soil pH range was 4.45-6.35 at the end of the experiment. Our findings support the previous finding of Colombo et al. (2014) who suggested that acidic condition helps mobilising Fe oxide and produces more extractable Fe. Production of siderophores and plant exudates by microbes can lead to solubilisation of Fe oxide (Colombo et al., 2014). The effect of Fe oxide+ OM was significant on the concentration of extractable Fe. However, the concentration of extractable Fe increased with an increase in Fe oxide level regardless of any level of OM treatment. Therefore, we assume OM had little or no effect on mobilising more extractable Fe. Although there are many observations regarding Fe oxide mobilisation due to the interaction of poorly crystalline Fe oxide + OM, in our experiment the concentration of extractable Fe did not significantly increase due to the interactive effect of Fe oxide + OM by the end of the experimental time. We assume long term organic amendment addition would make a significant difference in the concentration of extractable Fe in soil (Colombo et al., 2014; Wen et., 2019)

5.4.5. Changes in the masses of S+A fractions and the concentration of organic carbon in S+A fractions (>250 **µm**)

The masses of S+A fractions increased by the end of 8 weeks regardless of any treatments. In general, watering the wheat plants twice a week created an alternate wetting and drying cycle in the soil which had a positive impact on macro-aggregation (Rahman et al., 2018). Furthermore, wheat plants root growth and intensive root channels promoted macro-aggregate formation by entangling soil particles around the root channels as well as cementing soil particles together through the gluing effect of root exudates (Tisdall and Oades, 1982; Millar and Jastrow, 1990).

Our experimental soil was a sandy clay loam soil with a low to medium clay content. Generally, temperate soils contain a mixture of layer silicates such as illite, chlorite, and kaolinite (Robert et al., 1991; Wilson, 1999) with a predominance of phyllosilicates in most agricultural soil (Virto et al., 2008). According to the information from a previous research worker (Caio Fernandes Zani, Postgraduate Researcher, School of Natural and Environmental Sciences, Newcastle University, 2019), the experimental soil had a presence of kaolinitic (1:1 type) clay minerals. In soil samples that were amended with only Fe oxides (no OM), the masses of sand and stable aggregate (S+A) fractions significantly increased with increasing Fe oxide treatments by the end of the experiment. In soil samples that were amended with different combinations of Fe oxide + OM, the masses of S+A fractions did not significantly increase by the end of the experiment. We assume soils that were amended with only Fe oxides had higher potential to cause a significant increase in masses of S+A fractions by the end of incubation period than that of the soils which were amended with both Fe oxide +OM. Our result supports the previous findings that soil containing variable charge clay minerals and amorphous Fe oxides have higher potential to form stable macro-aggregates with low OM content compared to the soil with high OM content (Oades and Waters, 1991; Denef et al., 2002).

Furthermore, masses of S+A fractions increased with an increasing level of OM amendments. We assume OM caused increased microbial activity in the soil, resulting in the production of microbial metabolites which contributed to macro-aggregation (Nicolas et al., 2013). Moreover, the addition of OM as a source of N fertiliser promotes the growth of extraradical arbuscular mycorrhizal fungi (AMF) hyphal length which could be a possible reason behind increased soil macro-aggregates due to OM treatments (Wilson et al., 2009).

There is a significant positive correlation ($P<0.001$, $R= 0.367$) between the concentration of extractable Fe oxide and the mass of water-stable macro-aggregates (Table 5-22.).

At the end of 8 weeks, the concentration of organic carbon in S+A fractions increased with increasing Fe oxide treatment. Both OM treatment and the interaction of OM + Fe oxide significantly affected the concentration of C after 8 weeks. There was a positive significant correlation ($P<0.001$) between the concentration of C and the mass of S+A fractions. In addition, there was a significantly strong positive correlation ($P<0.001$, $R= 0.824$) between the total amount of organic C in S+A fractions and the mass of S+A fractions.

We found the concentration of organic carbon significantly increased due to the OM amendment which could be the direct input of organic carbon from the OM and an indirect increase in C from the net primary production (Wen et al., 2019). Furthermore, in temperate soils, OM intercalates into the interlayers of expandable phyllosilicates which could be a reason for the increased amount of stabilised organic carbon (Fernández-Ugalde et al. 2013). Our result showed that by the end of 8 weeks the concentration of carbon in stable aggregates was significantly greater due to the interactive effect of Fe oxide + OM compared to their individual effect. Research suggests that organo-mineral association is a dominant mechanism for soil organic carbon sequestration. The interaction between poorly crystalline Fe oxide and OM, not only depends on the reactive surface area of Fe oxide but also the composition of organic adsorbate, amount of protonated hydroxyl groups at different pH and the amount of OM input (Kaiser and Guggenberger, 2003; Eusterhues et al., 2005b; Kleber et al., 2005). According to Zimmermann et al., (2007) sand and stable aggregate fractions (S+A) represent the microbial biomass carbon and humified organic carbon pool. We assume due to the preferential sorption of OM on Fe oxide surface, small organic molecules like humic acid and lignin were able to enter into the micropores (<2nm) of Fe oxide (Kaiser and Guggenberger, 2003) which could be one of the mechanisms behind an increased concentration of stable organic carbon. Likewise, higher carbon load with the presence of sufficient Fe oxide surface could explain the result that 12 t/ha OM + 2% Fe oxide had a higher concentration of carbon than that of 1.5, 3, and 6 t/ha Om within 2% Fe oxide treatment (Kögel-Knabner et al. 2008).

There was no correlation between the concentration of C in macro-aggregates (S+A fractions obtained from >250 µm fractions) and extractable Fe oxides ($P>0.05$) whereas there was a positive correlation between the concentration of carbon in micro-aggregates (rSOC fractions, 0.45-63µm) and extractable Fe oxides (Section, 5.4.8). The amount of total C (g) and the concentration of extractable Fe correlated better at micro-aggregate level (rSOC fractions, 0.45-63µm) ($P<0.01$; $R= 0.484$) than that in macro-aggregate level ($P= 0.0104$; $R= 0.295$) (Table 5-22.). This finding supports previous studies by Duiker et al. (2003). They suggested the amount of organic carbon in aggregates and the amount of poorly crystalline Fe oxides correlate better where there is a presence of a relatively lower amount of organic carbon. Micro-aggregates generally contain less amount of organic carbon than macro-aggregates (evident from Table 5-7 and Table 5-19). Therefore, there is a correlation between the concentration of carbon and the concentration of extractable Fe oxides at the micro-aggregate level but not at macro-aggregate level.

5.4.6. Changes in mass of POM

The amount of POM derived from both macro-aggregates (> 250 μm) and micro-aggregates (63-250 μm) did not differ significantly due to any of the treatments except time. After 8 weeks the masses of POM fractions in macro-aggregates were significantly higher than that of before 8 weeks. The masses of POM increased due to the accumulation of undecomposed root and wheat plant fractions in macro-aggregates (Halder and Guggenberger, 2005). In addition, density fractionation of macro-aggregates helped to recover loosely bond organic particles from the macro-aggregate surface (Halder and Guggenberger, 2005).

The masses of POM derived from micro-aggregates (63-250 µm) was lower at the end of the experiment compared to the beginning. The reason behind this could be occlusion of POM in micro-aggregate or association with mineral fractions and formation of organo-mineral complex, resulting in POM becoming more resistant to be recovered by density separation (Golchin et al., 1994b; Yamashita et al., 2006).

5.4.7. Changes in mass of S+A (63-250 µm) and S+C (0.45-63 µm) fractions:

Our result showed that none of the treatments significantly affected the masses of sand and stable aggregate (S+A) fractions (obtained from 63-250µm fractions). The masses of silt + clay (S+C) fractions (0.45-63 µm) significantly decreased after 8 weeks regardless of any treatments. The reason behind the decrease in S+C fractions could be explained by the fact that silt and clay fractions bound up in macro-aggregates or participated in the formation of rSOC fractions, resulting in an increased mass of S+A fractions (Section 5.3.5.1) and rSOC fractions (Section 5.3.5.7) and a decrease in mass of S+C fractions (Wei et al., 2016). The reason behind little or no effect of given treatments on the measured fractions could be explained by the fact that soil is a heterogeneous system. Research suggests that spatial variability of soil microbes and their enzyme production as well as the heterogeneous distribution of OM significantly affects the interaction between OM and mineral surfaces (Ettema and Wardle, 2002, Kandeler and Dick, 2006 and Fry, 2007), thereby our result showed a high level of uncertainty in the masses of S+A and S+C fractions.

5.4.8. Changes in masses of rSOC fractions and the concentration of organic carbon in rSOC fractions

In this experiment chemical degradation of 0.45-63 µm fractions was carried out by using an oxidising agent. This oxidation process preferentially removes the labile organic carbon in soil and leaves the mineral associated OM (*Mikutta* et al., 2005) which is considered as resistant soil organic carbon fraction. Our result showed Fe oxide treatment significantly increased the masses of rSOC fractions and the concentration of organic carbon in rSOC fractions by the end of 8 weeks. However, the effect of OM and its interaction with Fe oxide over time was not significant.

Oxidation resistant organic carbon fraction is produced by its interaction between Fe oxide and clay mineral (Eusterhues, 2005a). Research suggests that temperate agricultural soils contain a greater amount of free clay fraction (<2µm) than clay-sized aggregates which facilitates the formation of kaolinite-oxide micro-aggregates. (Chenu & Plante, 2006, Virto et al. 2008). In addition, at the beginning of our experiment soil pH was 5.28-6.24. We assumed this slightly acidic condition favoured the ligand exchange reaction between the carboxylic group of aliphatic organic matter and the positively charged single coordinated hydroxyl group on Fe oxide surface (Eusterhues, 2005b), resulting in the formation of organo-mineral complex and the increased concentration of organic carbon in rSOC fractions.

There is a significant positive correlation between the amount of Fe oxide and the concentration of resistant organic carbon ($P<0.01$, $R=0.418$). This finding supports the previous study by Mikutta and Kaiser, (2011) where they found that an increase in Fe oxide content increases the amount of resistant soil organic carbon in different soils.

5.4.9. Changes in the concentration of dissolved organic carbon

The concentration of dissolved organic carbon significantly reduced due to Fe oxide treatment by the end of 8 weeks period. This result could be explained by the adsorption or precipitation of organic molecules on mineral surfaces, or the formation of the organo-mineral complex (Kögel-Knabner et al., 2008; Kleber et al., 2015). Research suggests that DOC rich in aromatic compounds or carboxyl groups are preferentially adsorbed onto Fe (hydr)oxides via ligand exchange or co-precipitation (Yost et al., 1990; Gu et al., 1995; Kaiser et al., 1996).

The interactive effect of OM + Fe oxide + time was not significant on the concentration of dissolved organic Carbon. In contrast, previous studies suggested that organically amended soil provides vigorous mobilisation of poorly crystalline Fe oxide and help co-precipitation or adsorption of DOC on mineral surfaces (Wen et al., 2019). We assume Fe oxide and OM interaction would make a significant reduction in DOC in long term organic amendment in soil (Wen et al., 2019)

5.5. Conclusion

This experiment met our hypothesis no (1) & (2). Fe oxide amendment in soil caused an increase in macro-aggregate (S+A fractions obtained from >250 µm fraction) formation. In addition, the effect of Fe oxide addition on macro-aggregate formation was further backed up by the effect of plant roots and organic matter. At micro-aggregate level sand and stable aggregate fractions (63-250 µm) were not affected by Fe oxide treatments but the amount of resistant organic carbon fractions (0.45-63 µm) increased due to Fe oxide treatments. Resistant organic carbon fractions represent the mineral associated organic carbon fractions. Therefore, plant root or OM had relatively less effect on the formation of rSOC fractions which evidencing the particular role of Fe oxide on micro-aggregate formation. Fe oxide treatment reduced the concentration of dissolved organic carbon (a biologically available form of organic carbon) and increased the concentration of organic carbon in solid aggregate fractions (S+A fraction >250 and rSOC fractions 0.45-63 µm). Our experiment did not fully satisfy our hypothesis no (3). There were many significant interactions of Fe oxide + OM but the interactive effect of Fe oxide +OM did not cause any significant differences in masses of aggregate fractions and the concentration of organic carbon in aggregate fractions over the incubation period except for the concentration of organic carbon in S+A (>250 µm). As we have already observed that the interactions between Fe oxide and OM were significant, we assume lengthening the experimental time might make a significant difference in stable aggregate formation as well as the concentration of stable organic carbon in aggregates. Future research scope and limitations of our current work are further discussed in Chapter 6.

5.6. References

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Chapter 6

Conclusion

6.1. Conclusion

The aim of this thesis was to investigate the role of Fe oxides on stabilising soil organic carbon. We found that Fe oxides can reduce the release of dissolved organic carbon from soils (Chapter 4& 5). Fe oxide amendment caused an increase in the amount of stable macro-aggregates and the concentration of organic carbon in macro-aggregates (Chapter 5). In micro-aggregates, Fe oxide increased the amount of resistant organic carbon fractions and the concentration of organic carbon in the resistant organic carbon fractions. In terms of the joint contribution of Fe oxide + OM, only the concentration of organic carbon in macro-aggregates significantly increased over time due to the interactions of Fe oxide + OM. We assume the duration of our incubation study was not long enough to show a significant change in the masses of micro-aggregates or the concentration of carbon in micro-aggregates due to the interactive effect of Fe oxide + OM.

Nevertheless, our experiments certainly reveal that Fe oxide (ochres) amendment in soil could be a useful tool to increase soil aggregate formation and storage of stable organic carbon in soil. However, several physical, chemical and biological processes simultaneously contribute to the stabilisation of soil organic carbon which made it difficult to specify the contribution of Fe oxide in SOC stabilisation. Identifying the actual bonding mechanism in the organo-mineral complex could show a better insight into our results. Furthermore, under field studies, our result might vary to some extent. In agricultural field, aggregate turnover promotes rapid mineralisation of SOC and a substantial loss of easily decomposable carbon (Six et al., 1998) (discussed in Chapter 2, Section 2.7). Our experiments could not reveal whether Fe oxide amendment protects aggregate turnover or not.

In field studies farming practices (liming and fertilisation), seasonal variation of litter fall or OM input control soil pH, ionic strength, the degree of humification of OM, quality of DOC and their release to soil solution, thereby the potential of Fe oxide to adsorb DOC varies (Kalbitz et al., 2000). According to Jardine et al. (1990), heavy rainfall causes a rapid transport of DOC from surface to subsurface horizon which results in reduced contact time between DOC and mineral surface.

Based on the current findings and limitations of our work we recommend further laboratory study accompanied with a field study with an aim to identify and quantify the actual bonding in organo-mineral complex as well as the effect of Fe oxide amendment on soil aggregate turnover time, which in turn would provide better information on the role of Fe oxides in soil organic carbon preservation.

In order to identify the bonding mechanism in organic matter-Fe oxides mineral interactions as well to quantify the binding strength of organo-mineral complex, studies could use the experimental technique called ‘Dynamic Force Spectroscopy’ (DFS) described by Newcomb et al. (2017). In DFS technique, organic molecules with known functional groups are forced to interact with the mineral surface and the strength of bond between the organic functional group and the mineral surface could be quantified (this study can be carried out in a mimicked natural soil environment such as soil pH, ionic strength). It provides numerical value for the free energy of binding in organo-mineral complex which is a representative of the type of bond. For the experimental setup of DFS measurement, we could use the common functional groups of soil organic matter.

The effect of Fe oxide amendment on soil aggregate turnover time could be achieved by combined isotopic tracer technique described by Peng et al., (2016) incorporation with the current soil fractionation method used in this thesis (Chapter 5). This technique determines aggregate turnover time in a controlled incubation study in the laboratory where four different naturally occurring aggregate size fractions were labelled with different rare earth oxide (REO) tracers and some isotopically labelled carbon, then mixed to form a composite soil sample. The shift of aggregate size fraction to the neighbouring aggregate size was measured at 0,7,14 and 28 days after incubation (achieved by wet sieving of aggregate fractions followed by the measurement of tracer element in each aggregate fractions). We recommend for the future study, conducting a long term field experiment where soils could be amended with Fe oxides and without Fe oxides (control). With a regular time interval, soil samples from the experimental field would be collected for the current fractionation (used in Chapter 5) and the shift of aggregate size fractions could be determined by the combined isotopic tracer technique mentioned above. Comparisons between the changes in aggregate turnover time in aggregate size fractions obtained from Fe oxide amended soils and the controls soils could help answering the question that how Fe oxide amendment affects soil aggregate turnover.

Furthermore, research considering the dynamics of DOC release into the soil would show a better aspect of our current adsorption study. We recommend measuring the concentration of DOC with varying soil depth with seasonal variation in a long term field study. Changes in concentration of DOC with depth and seasonal variation could track the mobility of DOC in soil.

6.2. References

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