THE UNIVERSITY OF
SYDNEY

FINDING AN EFFICIENT METHOD TO MEASURE SOIL CARBON POOLS

Meragal Pedige Nilusha Kumari Henaka Arachchi

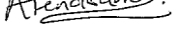
A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

August 2014

Faculty of Agriculture and Environment
The University of Sydney
New South Wales
Australia

Certificate of Originality

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Signature: 

Author Name: Meragal Pedige Nilusha Kumari Henaka Arachchi

Date: 29/08/2014

Thesis Summary

Primarily, this thesis addresses the need for a cost and time efficient methodology to quantify both chemically and physically stabilized soil organic carbon (SOC) fractions. The time and cost saving would be gained if the need to physically and chemically separate soil samples to measure carbons of different fractions could be reduced. Some progress has been made by quantifying SOC using spectroscopy techniques (NIR and MIR) in place of combustion, which both give quantitative and qualitative (organic functional groups) measurement for SOC. What remains is to see if the comprehensive data that can be gained from the spectroscopic techniques can be used to quantify and characterize SOC in soil fractions while using minimal or no physical and/or fractionation pretreatments.

Chapter 2 of this thesis is a comprehensive review of the literature focusing on soil C pools and significant aggregate theories currently developed. From the literature, it is apparent that soil aggregation is mediated by SOC and aggregate fractions of various sizes are stabilized by a range of SOC functional groups. At the same time SOC and aggregation models were being developed SOC pools were also being defined to populate soil carbon turnover models (e.g. Roth-C). Only recently, has research been undertaken which attempt to combine soil aggregation with soil C turnover models. A limitation to using these models is the time taken to separate aggregate and carbon fractions and the review of the literature indicates that using spectroscopic techniques may be a solution to this.

In Chapter 3 both NIR and MIR spectroscopy are evaluated to predict the total organic carbon (TOC) contained within macroaggregates, microaggregates, and organo-mineral associations. To do this soil samples collected from three bio regions of New South Wales, Australia and fractionated using a wet sieving procedure. Ground aggregate fractions and bulk soil samples were scanned using NIR and MIR spectrometers. The TOC of aggregate fractions predicted using the Cubist model from bulk soil NIR and MIR spectra. The cross-validation results confirmed that the use of MIR to predict TOC

aggregate fractions is more accurate than for NIR. Absorption differences of mean NIR and MIR spectra of aggregate fractions and Cubist model used wavelengths showed specific functional groups related to each aggregate fraction. The observed specific functional groups were compatible with the aggregate formation and SOC stabilization described by aggregate hierarchy model.

According to the aggregate hierarchy model, each aggregate fraction is stabilized by different SOC fractions. Thus, Chapter 4 focuses on quantifying different SOC fractions stabilized within macroaggregates, microaggregates, and organo-mineral associations using MIR spectra. Five soil carbon fractions were separated from the bulk soils, macroaggregates, microaggregate and organo-mineral associations using a combined physical-chemical fractionation procedure. Macroaggregate fractions were dominated by LF-POM fraction and microaggregates and organo-mineral associations were significantly associated with stable SOC fractions (S+C and RSOC). Poor predictions were observed for resistant soil organic carbon (RSOC) and dissolved organic carbon (DOC) while other fraction where predicted accurately with Cubist/MIR models for aggregate associated carbon fractions.

Changes in soil aggregation and soil C turnover are determined by different environmental factors such as temperature and moisture. While temperature is one of the most widely studied environmental factor in relation to soil carbon turnover, there are fewer studies on the effect on aggregate protected soil carbon. Therefore, Chapter 5 focuses on assessing the temperature sensitivity of soil aggregate associated C and spectral changes over time due to soil C mineralization of different land use systems. Changes of the aggregate associated C and SOC fractions over 180 days incubated soils were observed and the models developed in Chapter 4 were used to predict changes in carbon fractions during the incubation. The macroaggregate associated carbon depleted significantly compared to the microaggregate and organo-mineral associated C. Light faction particular organic matter (LF-POM) lost more C as it is highly associated with the macroaggregate fraction. The spectral changes clearly showed the decrease of specific functional groups associated with aggregate fractions and SOC fractions.

The research findings of the Chapter 3 to 5 synthesizes in Chapter 5 and implies research gaps identified in Chapter 2. The investigations carried out in Chapter 2 to 5 are giving directions for future investigations discussed in Chapter 6.

TO MY BELOVED GRAND-MOTHER

Who gave me the first lesson of life

Acknowledgement

First, I wish to sincerely thank my supervisors, Damien Field and Alex McBratney for their inspiring suggestions, excellent support and encouragement given in all phases of my research. It was such a privilege to have an opportunity to work with Damien and Alex and I have no words to explain the value they brought in to my study through their guidance and professional inputs. With their extremely busy schedules the time they spent on my study, effort, commitment and dedication they showed was quite remarkable. They always understood my problems and were right behind me providing constant support, guidance and supervision to overcome all obstacles I faced during my study. Without their support I would never be able to reach this memorable and happy milestone in my life submitting my PhD.

Secondly, I would like to thank my husband, my little daughter, my parents and my in-laws for the great support that they have given me during past 3 1/2 years. They not only released me from the responsibility of looking after my little daughter but also from all responsibilities at home allowing me to dedicate my full time and effort on my PhD. I'm really fortunate to have such a wonderful family with great support otherwise completing my study on time with all required laboratory experiments would be an absolute challenge.

I would like to acknowledge the University of Sydney for awarding me this International Scholarship (USydIS) to for my postgraduate studies. It was one of my dreams to study in University of Sydney and USydIS helped me fulfilling my dream. Also I would like to extend my appreciation to other faculty teaching staff Budiman Minasny, Balwanth Singh, Feike Dijkstra, and Claudia Keitel who helped me in many ways in different stages in this study.

I’m expressing my gratitude to Michael Zimmermann (University of Natural Resources and Life Sciences Vienna) who sent me some important advices about the fractionation procedure which I followed in this thesis.

Sampling from bioregions of New South Wales and Hunter Valley area was something I may not able to do by myself and I appreciate the assistance Kanika Singh and Brendan Malone gave me in collecting these samples. Moreover Kanika was the person who was helped me from my first day at the university and I really admire all the support she gave me during all this time.

Thank you Mario Fajardo, Sebastian Campbell and José Padarian for helping me to use R software and statistical analysis. I would also like to thank Yunying Fang for sharing her knowledge for setting up the incubation experiments, Yolima Carrillo and Hero Tahaei for providing transport for me when I need to travel Cobbitty for isotope and TOC analysis, Loraine Watson, Michael Turner, Iona Gyorgy, Nile Wilson and Ivan Desailly of Faculty of Agriculture and Environment for providing me Technical support.

Last but not least my sincere appreciation goes to Senani Karunaratne, Cheryl Poon, Robert Pallasser, Nirmala Liyanage, Nathan Odgers, Zhaolong Zhu, Waqar Ahmad, Maryam Montazerolghaem, Sabina Yeasmin, Jason Morris and Chamali Nagodvitane those who have contributed time and effort in many ways to make this research effort a reality.

Table of Content

List of Table

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Terminology

Soil carbon (SC) - Carbon held within the soil

Soil organic carbon (SOC) - Carbon associated with soil organic matter.

Soil carbon fractions - Measurable organic matter components

Soil carbon pool - Theoretically separated, kinetically delineated components of SOM

Soil aggregates - 'Clumps' of soil particles that are held together by moist clay, organic matter (such as roots), by organic compounds (from bacteria and fungi) and by fungal hyphae

Macroaggregates - Soil aggregates size > 250 µm

Microaggregates - Soil aggregates size 63 - 250 µm

Organo-mineral associations - Soil aggregates size < 63 µm

Aggregate carbon - Carbon associated with soil aggregates

Macroaggregate C - Carbon associated with macroaggregates

Microaggregate C - Carbon associated with microaggregates

Organo-mineral associated C - Carbon associated with organo-mineral associations

Bulk soil - Unseparated soil into SOC fractions or aggregate fractions

Chapter 1: General Introduction

1.1. Introduction

Soil organic matter (SOM) is recognized by scientists as a major factor controlling the capacity of soil resources to deliver agricultural and environmental services, as well as sustain human societies at both the local and global scale (Manlay et al., 2007). A large number of research projects have been initiated globally where soil C is a key component. Currently, over 1,000 articles are being published annually in peer-reviewed journals, increasing at about 10% per year (Hartemink et al., 2014). SOM is a heterogeneous complex compound consisting of various organic functional groups that are physically protected and/or stabilized by specific chemical binding mechanisms resulting in different certain turnover rates. These are conceptually divided into carbon pools based on their biological stability (labile, stabile, refractory and inert), decomposition rate (fast-active, slow-intermediate and very slow/passive/inert), or turnover time (short, long, very long) (Krull et al., 2003). Each pool of SOM has a specific function and most of these are due to the relative stability and biological availability of the pools.

The amount and distribution of SOM fractions defined by different turnover rates can be estimated by using one of several soil organic carbon (SOC) models (Zimmermann et al., 2007). These models also provide means for evaluating the relative importance of mechanisms that stabilize carbon over a range of timescales and for predicting large-scale terrestrial C dynamics in response to climate environmental and management changes (Lawrence et al., 2009). Many SOC models that have been develop over the last 40 years attempt to overcome the problem posed by SOC heterogeneity by defining a number of distinct pools (Krull et al., 2003). To achieve this they have adopted of a range of various fractionation schemes to isolate biological meaningful SOC fractions to populate simulation models such as CENTURY, Roth- C, CANDY, DAYCENT, and DAISY (Coleman and Jenkinson, 1996; Franko et al., 1996; Mueller et al., 1996; Parton, 1996). Roth-C and the CENTURY model have explained the relative amount of SOC fraction changes with time by using various assumptions accounting for the combined

chemical recalcitrance and physical protection of the SOC (Coleman and Jenkinson, 1996; Parton, 1996).

The fact that these models do not discriminate between these two mechanisms affecting the longevity of SOC means that the SOC is not routinely spatially quantified in relation to any aggregate fraction within a hierarchy of aggregates, as they were defined by Tisdall and Oades (1982) or Six et al. (2000). According to the aggregate hierarchy model (Jastrow and Miller, 1998) the smallest aggregates are composed of organo-mineral associations which are then bound together with bacterial and fungal debris to form microaggregates (< 250 μm). The clustering of these small aggregates forms macroaggregates (> 250 μm). Microaggregates are stabilized mainly by persistent binding materials (aromatic humic materials) and macroaggregates by transient (polysaccharides) and temporary agents (roots, fungal hyphae, bacterial cells and algae). There is little evidence to show how these SOC pools migrate through the various conceptual organic carbon pools over time and how the redistribution within these pools affects changes in soil structure. To address this, both Struc-C and AggModel were proposed (Malamoud et al., 2009; Segoil et al., 2013) which describe how the SOC influences the dynamics of soil structure, and consequently, soil physical behaviour. Since SOC is closely related to the formation of stable soil aggregates (Tisdall and Oades, 1982) being able to quantify a relationship between aggregate formation and changes in soil carbon may prove useful to predict and numerate the associated soil structural changes.

It has been shown that there is a direct link between soil aggregation and C sequestration (Lal et al., 1998), which is attributed to the biochemical and physical stabilization of SOC when bound to mineral surfaces and/or occluded in soil aggregates (Krull et al., 2003). Therefore, understanding how an aggregate stores and interacts with SOC is fundamental to developing management strategies toward the enhancement of C sequestration at regional and global scales (Bronick and Lal, 2005). This is based in the premise that well aggregated soils consist with more physically protected showing lower C mineralization rates compared to disaggregated soils (tilled soil). Previous literature has proved that different land use systems have different levels of aggregate formation

abilities depending on their management condition (Six et al., 2000). Forest and grassland ecosystems were shown to consist of better aggregated soils than cultivated lands. This proves forest and grassland soil having a higher C-sequestration potential compared to cultivated land due to the physical protection of SOC (Bongiovanni and Lobartini, 2006). The SOC decomposition rate also depends on climatic factors (soil moisture and temperature), soil chemical factors (pH and oxygen availability), mechanisms of physical protection and clay content and mineralogy of the soil (Paustian et al., 1997). Thus, temperature is a main climatic factor and greatly affects to global C cycle and climatic changes (Davidson and Janssens, 2006). Most of previous incubation studies have found higher temperature sensitivity for stable SOM in comparison to labile SOM (Conant et al., 2011). However, temperature sensitivity of aggregate protected SOC was rarely tested.

Various methods have been proposed to separate soil samples into fractions with distinct chemical and physical characteristics corresponding to different stabilizing mechanisms and soil functions (von Lützow et al., 2007). These procedures are time consuming and expensive and therefore quite often assessments of soil C in both research and industry do not consider SOC fractions. Recently, non-invasive techniques such as infrared (IR) were proven to be effective when estimating soil properties with very short measuring times and comparatively lower costs than conventional techniques. For this reason near infrared (NIR) and mid infrared (MIR) spectroscopy are considered as alternatives to conventional analytical methods (Viscarra-Rossel et al., 2006). Efforts have been made to use IR to estimate the SOC of physically separated carbon fractions, but not using IR scan of the whole soil to infer or estimate aggregate fractions. While there may be some time saving in using IR compared to oxidation techniques there is little advantage unless the need to overcome the physical separation of soil samples. It is soil C data that is needed to populate soil C turnover models, such as Roth-C, and the newly proposed physical models, Struc-C and AggModel.

1.2. Objectives

1. To quantify soil aggregate associated SOC using near and mid infrared (NIR and MIR) spectroscopy.
2. To investigate the potential to measure aggregate associated SOC fractions using MIR spectroscopy.
3. To estimate the changes of the aggregated C and SOC fractions in incubated soils using MIR spectroscopy.

1.3. References

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma. 124, 3-22.

Bongiovanni, M.D., Lobartini, J.C., 2006. Particulate organic matter, carbohydrate, humic acid contents in soil macro- and microaggregates as affected by cultivation. Geoderma. 136, 660-665.

Coleman, K., Jenkinson, D.S., 1996. RothC-26.3-a model for the turnover of carbon in soil. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models, NATO ASI Series, Springer, Berlin. pp237-246.

Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R., Kirschbaum, M.U.F., Lavallee, J.M., Leifeld, J., Parton, W.J., Steinweg, J.M., Wallenstein, M.D., Wetterstedt, J.Å.M., Bradford, M.A., 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology. 17, 3392-3404.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature. 440, 165-173.

Franko, U., 1996. Modelling approaches of soil organic matter turnover within the CANDY system. In: Powlson, D.S., Smith, P., Smith, J.U., (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-Term Datasets. NATO ASI Series I, pp47-254.

Hartemink, A.E., Lal, R., Gerzabek, M.H., Jama, B., McBratney, A., Six, J. Tornquist, C.G., 2014. Soil carbon research and global environmental challenges. PeerJ Pre Prints 2:e366v1 http://dx.doi.org/10.7287/peerj.preprints.366v1

Jastrow, J.D., Miller, R.M., 1998. Soil aggregate stabilization and carbon sequestration: feedbacks through organo-mineral associations. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A., (Eds.), Soil Processes and the Carbon Cycle. CRC Press, Boca Raton. pp207-223.

Krull, E.S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Functional Plant Biology. 30, 207-222.

Lal, R., 2008. Carbon sequestration. Philosophical Transactions: Biological Sciences. 363, 815-830.

Lawrence, C.R., Neff, J.C., Schimel, J.P., 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biology and Biochemistry. 41, 923-1934.

Malamoud, K., McBratney, A.B., Minasny, B., Field, D.J., 2009. Modelling how carbon affects soil structure. Geoderma. 149, 19-26.

Manlay, R.J., Feller, C., Swift, M.J., 2007. Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems and Environment. 119, 217-233.

Mueller, T., Jensen, L.S., Hansen. S., Nielsen, N.E., 1996. Simulating soil carbon and nitrogen dynamics with the soil-plant-atmosphere system model DAISY. In: Powlson, D.S., Smith, P., Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of Factors Controlling Soil Organic Matter Levels in Great Plains Grasslands1. Soil Science Society of America Journal. 51, 1173-1179.

Parton, W.J., 1996. The CENTURY model. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets. NATO ASI Series I. Springer-Verlag, Heidelberg, pp283-293.

Paustian, K, Collins H.P., Paul, E.A., 1997. Management controls on soil carbon. In: Soil Organic Matter in Temperate Agro ecosystems. Paul, E.A., Paustian, K., Elliott, E.T., Cole, C.V., (Eds.), CRC Press, Boca Raton, FL, pp15-49.

Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-Term Datasets. NATO ASI Series I. 38, 275-282.

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry. 32, 2099-2103.

Segoli, M., De Gryze, S., Dou, F., Lee, L., Post, W.M., Denef, K., Six, J., 2013. AggModel: a soil organic matter model with measurable pools for use in incubation studies. Ecological Modelling. 263, 1-9.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science. 33, 141-163.

Viscarra-Rossel, R.A., Walvoort, D.J.J., McBratney, A.B., Janik, L.J., Skjemstad, J.O., 2006. Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. Geoderma. 131, 59-75.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry. 39, 2183-2207.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J., 2007. Measured soil organic matter fractions can be related to pools in the Roth-C model. European Journal of Soil Science. 58, 658-667.

Chapter 2: Soil carbon pools and their fractionation procedures in relation soil organic matter turnover models.

2.1. Importance of measuring SOM

Over the last 300 years, the evolution of scientific knowledge of SOM has enabled an understanding of the mechanistic relationships between SOM in terms of fertility and sustainability long recognized in farming practices (Manlay et al., 2007). In early 1700's, the concept of SOM first was described as a "humic" substance, and subsequently Wallerius (1761) defined humus in terms of decomposed organic matter. Even today, humus is recognized as the ''non-living, finely divided organic matter in soil, derived from microbial decomposition of plant and animal substances' (Encyclopaedia Britannica, 1990).

During the humic period (**Table 2.1**) use of animal manures was identified as an important component which maintaining soil fertility (Lawes, 1861). The understanding of SOM and soil biological functioning during the mineralist period was improving and it had a great impact on the rise of new mineral-based (nitrogen, phosphorous and potassium) cropping systems. The green revolution (1940-1960 introduced a huge fertilizer application to soils for the production of new cultivars of rice, wheat and maize (Pinstrup-Anderson and Hazell, 1985). The green revolution gave rise to an opposition to organic farming practices and resulted in environmental degradation and the loss of ecosystem services. Consequently at the end of the ecological period, 'sustainable agriculture' appeared as an important concept. and SOM has been gaining recognition as an ecosystem component and an indicator for soil quality and agro ecosystems fertility (Manlay et al., 2007).

Table 2.1. Soil organic matter concepts defined using scientific knowledge and related management practices over the past 300 years

Time	Scientific theory and knowledge	Management practices
Humic period (1700-1840)	Humus in the form of soluble carbon and directly assimilated by plants	Mineral (N, P, K) and organic fertilization are important for small and large scale farming
Mineralist period (1840-1940)	SOM controls physical and chemical properties of soil	Intensified cropping systems with chemical fertilizers
Ecological period (1940-2000)	SOM is an ecosystem component and conceptualized as a mixture of fractions (soil carbon pools)	Organic farming, Composting, mulching, agro-forestry, cover crops, integrated nutrient management

SOM influences many soil functions and occupies a key position in the global carbon cycle (Lal, 2004). At present SOM is recognized as a major factor controlling the capacity of soil resources to deliver agricultural and environmental services and sustain human societies at both local and global scales (Feller et al., 2012). At the global scale, it is an essential component in ecosystems, influencing atmospheric chemistry and the world's climate through the global biogeochemical cycling of C (**Table 2.2**) (Chabbi and Rumpel, 2009). At the farm scale, productivity is depend on the cycling of SOM through the action of decomposers (mainly bacteria and fungi), which mineralize organic compounds and release nutrients necessary for plant growth (Gregorich et al., 1994). Therefore, productivity and sustainability of any agro-ecosystem focuses to maintain and improve SOM content (Gregorich et al., 1994).

Table 2.2. Functions of SOM at different scales

Scale	Function of SOM
Global scale	Carbon sequestration - moderation of climate through sequestration of atmospheric CO_2 into stable SOM components with a long residence time and the ability to oxidize CH_4.
Regional scale	Enhancement of soil quality and food security - act as a natural chelating material
Farm scale	Maintain the soil fertility and productivity - providing nutrients and habitat for soil organisms, enhance the soil structure, enhances water holding capacity, cation exchange capacity and main store of many key nutrients

2.2. Soil organic matter pools

Use of new techniques such as chromatography, analytical pyrolysis, nuclear magnetic resonance and isotopes confirmed SOM to be a heterogeneous compound comprised of complex natural materials and a mix of molecules of varying polymericity and aromaticity (Skjemstad et al., 1998; Stevenson and Cole, 1999). Thus, SOM may range in size and complexity from simple monomers or organic acids to mixtures of complex biopolymers aggregated together in the form of cellular debris (Baldock and Skjemstad, 2000). Inherent chemical complexity of SOM and different biochemical and physical stabilization processes are resulting in a range of conceptual SOM pools with turnover rates ranging from minutes to millennia (Six et al., 2002a; Jenkinson, 1987). These pools are defined by their different turnover times, their pool sizes, and how the SOM is protected and stabilized. This has resulted in different terminologies being used to describe these pools. The nomenclature and description of these pools are listed in **Table 2.3.** Depending on authors overlapping terminology is often applied to fractions and

pools that are not closely related and this has led to confusion (Wander, 2004) SOM commonly classified into three pools i.e. active pool, recalcitrant pool and inert pool.

Table 2.3. Soil Organic Matter Pools defined by different authors

Author	SOM Pool	Properties	Turnover time
Parton et al. (1987)	Active	Microbes and microbial products	Months to few years
	Slow	Resistant plant materials ex- lignin	20-50 years
	Passive	Physically and chemically stabilized SOM	400-2000 years
Coleman and Jenikson (1999)	Decomposable plant materials	Plant C from crop residues	*
	Resistant plant materials	Plant C from crop residues	*
	Humified	*	50 years
	Biomass	Microbes and microbial products	*
	Inert	Biologically not decomposable	10,000 years
Six et al. (2002)	Protected SOM	SOM protected by biochmical proceseses, microaggregates and silt and clay	*
	Unprotected SOM	SOM as plant or nutrient source	*
Wander (2004)	Labile or active	Equated with material of recent origin or embodied living components of SOM	Days to few years
	Slow or intermediate	Partially decomposed residues and decay products	Few years to days
	Recalcitrant, passive, stable, and inert	Recalcitrance because of biochemical characteristics and/or mineral association	Decades to centuries

Author	**SOM Pool**	**Properties**	**Turnover time**
Trumbore (2009)	Active pool	Root exudates, rapidly decomposed components of fresh plant litter	Years
	Intermediate or slow pool	Intermediate between the fast and slow-cycling pools	Decades
	Passive pool	Stabilized organic matter due to chemical or physical mechanisms	Centuries to millennia
Baldock (2007)	Surface plant material	Plant material residing on the surface of the soil, including leaf litter and crop/pasture material	Days to years
	Buried plant material	Plant material greater than 2 mm in size residing within the soil	Days to years
	Particulate SOM	-decomposed organic material smaller than 2 mm and greater than 50 µm in size	Days to years
	Humas	Well decomposed organic material smaller than 50 µm in size that is associated with soil particles	Years to decades
	Resistant SOM	Charcoal or charred materials that results from the burning of organic matter (resistant to biological decomposition)	Decades to thousands of years

* source did not provide the information

2.2.1. Active SOM

The active pool of SOM is often termed the labile pool. This pool is the easily mineralizable pool of SOM with a turnover time ranging from days to few years (Wander, 2004). The labile pool is chemically degradable and physically accessible by soil microbes (Zou et al., 2005). This pool plays an essential role in the short-term nutrient turnover in soil (Parton et al., 1994). The labile pool also acts as the fuel of the soil food web and therefore greatly influences nutrient cycles and many biologically related soil properties. This being the case, the active pool is identified as an indicator of changes in management-induced soil quality (Islam and Weil, 2000; Kennedy and Papendick, 1995). The active pool includes microbial biomass carbon, particulate organic matter, simple carbohydrates, amino acids and dissolved organic matter (Islam and Weil, 2000; Wander and Bidart, 2000).

2.2.1.1. Dissolved organic matter (DOM)

Dissolved organic matter is the most mobile fraction of SOM and can therefore reach almost all soil particles by diffusion and convection. DOM consists of SOM ranging from small-defined molecules to colloidal substances (Zsolnay, 1996), and explicitly is defined as < 0.45 µm in solution. DOM is an energy source for microbes and plays a significant role in biodegradation and decomposition processes (McDowell et al., 2006). In agricultural soils, DOM typically accounts for only 0.05-0.4% of SOC, in forest soils it is often in the range of 0.25-2% (Haynes, 2005). About 70% of DOM is extractable from old SOM pools (Kalbitz et al., 2003).

2.2.1.2. Microbial biomass carbon (MBC)

The soil microbial biomass is considered to be the chief component of the active SOM pool and regulates all SOM transformations (Smith and Paul, 1990). Generally, MBC represents between 0.3-7% of SOC (Wardle, 1992). In agricultural top soils MBC ranges around 0.3-4% and in forest ecosystems it is ranges from 0.5-0.9% in O-horizons to 1.6-

3.6% in A-horizons (Bundt et al., 2001). Changes in clay content, mineralogy, and vegetation can influence the proportion of MBC (Sparling, 1992). MBC is an acute component of SOM quality as it provides an indication of a soils' ability or capacity to store and recycle nutrients and energy (Gregorich et al., 1994). This fraction has a great impact on soil aggregation and on the characteristics (composition, origin) of different functional OM pools (Lundberg et al., 2001).

2.2.2.3. Particulate organic matter (POM)

Particulate organic matter pool is characterized by organic fragments with a recognizable cellular structure which are derived from any source but usually dominated by plant derived materials (Baldock and Nelson, 1999). Most of SOM that enters the soil is in a particulate form (particle size > 53μm) and is derived from plant debris (Wander, 2004). The initial composition of free POM in the soil is characterized by a high content of polysaccharides, typically found in fresh plant and microbial tissues (e.g. cellulose, hemicelluloses. chitin, peptiglycan), and a C/N ratios decrease to 12 or less (Boldock and Skjemstad, 2000). This pool consists of unprotected POM as 53-2000 μm sized, not contained within microaggregates and protected POM as 53-250 μm sized POM contained within microaggregates (Six et al., 2002a). POM is biologically and chemically active and is commonly used as an index of the labile SOM pool (Buyanovsky et al., 1994). This pool is important to microbial growth and nutrient supply, and suggests that it is closely related to biologically mediated C, N, and in some soils P availability (Gregorich et al., 1994; Hassink, 1995b).

2.2.2. Inert SOM

The inert pool (IOM), also known as passive pool (Krull et al., 2003), is not biologically decomposable and therefore has no decomposition rate. In models such as the Rothamsted C model (Roth-C), this pool is uncoupled from the other SOM pools, and is effectively a constant (Coleman and Jenikson 1996). Thus conceptually, IOM remains

unaffected by changes in climate, land use or management and whilst its size is of importance (Falloon and Smith, 2000). Since, IOM has no finite turnover time it is therefore conceivable this could, infinitely accumulate in the soil, but there can be loss due to vertical and lateral transport from the system.

The IOM pool includes highly carbonized organic materials such as charcoal, graphite and coal. Charcoal or black carbon originates from incomplete combustion of organic material and considered the most recalcitrant structure of SOC due to its high degree of aromaticity and highly condensed chemical structure (Derenne and Largeau, 2001). Although it's resistant to decomposition, charcoal has an estimated turnover time or residence time of 5000 to 10000 years (Skjemstad et al., 1998). As well as an important sink for atmospheric CO_2, in soils and sediments, it substantially contributes to the sorption of organic pollutants (Bornemann et al., 2007; Lohmann et al., 2005) and heavy metals (Hiller and Brümmer, 1997). It is also suspected to be involved in the stabilization of humus (Schmidt et al., 1999) and soil aggregation (Brodowski et al., 2006; Picollo et al., 1987).

2.2.3. Resistant soil organic matter (RSOM)

The resistant pool is often synonymously referred to as intermediate, slow recalcitrant or refractory (Fang et al., 2006; Krull et al., 2003). This pool has a long (10-100 years) turnover time and is coupled to other SOM pools (Kleber, 2010). RSOM is important for long-term C sequestration, sorption, CEC, and soil water-holding capacity (Wander, 2004). Stabilization of the SOC in this pool occurred due to the two main processes biochemical stabilization and physical stabilization (Six et al., 2002a). For example, the chemical complexity of the SOM affects the rate of the biological mineralization of SOM, and is defined as biochemical recalcitrance. The interaction of SOM with the mineral phase through organo-mineral complexes or occlusion in soil aggregates is defined as physically protection of SOM (Six et al., 2002a).

2.2.3.1. Biochemical stabilization

Stablization of SOM by biochemical recalcitrance is the inherent chemical and structural stability of biomolecules. This is influenced by several factors including chemical structure of SOC, soil environmental factors including temperature, moisture and aeration, and soil physical and chemical properties (Krull et al., 2003). Alkyl structures, especially the non-hydrolysable forms, are considered to be chemically stable due to their highly aliphatic nature. They consist of lipids (e.g. fatty acids, waxes, cutin and terpenoids), insoluble polyesters, and macromolecules synthesised by micro-organisms. Waxes, in particular cutin and suberin, are polymerising structures that are resistant to microbial attack. As an aromatic compound lignin is more resistant to decomposition than carbohydrates and together with alkyl carbon it is a very stable form of SOC. SOC containing alkyl and lignin-derived aromatic carbon are thought to have turnover times between 10s to 100s of years and assigned to the stable or passive pools (Coleman and Jenkinson, 1996). Derenne and Largeau (2001) found that of the seven major classes of supposedly refractory biopolymers investigated only thermally altered carbon showed the presumed relationship between being refractory (¼ insoluble and nonhydrolysable) in the laboratory and being able to persist in a soil environment.

2.2.3.2. Physical stabilization

The physical protection of SOM is defined as the interaction of SOC with the soil mineral matrix, which results in physical or chemical inaccessibility of soil carbon to decomposer organisms by the formation of closed environments or strong bonds (Krull et al., 2003). This can occur due to physico-chemical stabilization by adsorption and chemical binding of SOC onto mineral surface or the occlusion in soil aggregates isolating the carbon from microbial and enzymatic attack (Krull et al., 2003).

2.3. Soil aggregates and soil organic matter dynamics

In early 1900s, the major factors important for aggregate formation and stabilization were identified very little effort was made to develop theoretical frameworks of aggregate formation and the relationships between SOM. After 1950s, the link between soil biotic activity, SOM decomposition and stabilization, and soil aggregate dynamics has intensively been studied (**Figure 2.1**). Initially, these studies focused on understanding the inorganic interactions responsible for the formation and stabilization of the soil aggregates, but after the 1950's the role of SOM and living organic matter in aggregation formation and stabilization was increasingly studied.

The first aggregate-SOM conceptual model was proposed by Emerson 1959, which described

how a soil crumb consisted of domains of oriented clays and quartz particles. Edwards and Bremner (1967) proposed microaggregates are formed by bonding of C-P-OM clay sized units, where C: clay particle, P: polyvalent metal (Fe, Al, Ca) and OM: organo-metal complex, and are represented as [(C-P-OM)x]y. It is evident that the C-P-OM units are equivalent to the clay domains of Emerson. A significant milestone was in 1982, Tisdall and Oades proposed an aggregate hierarchy concept of aggregate-SOM interactions.

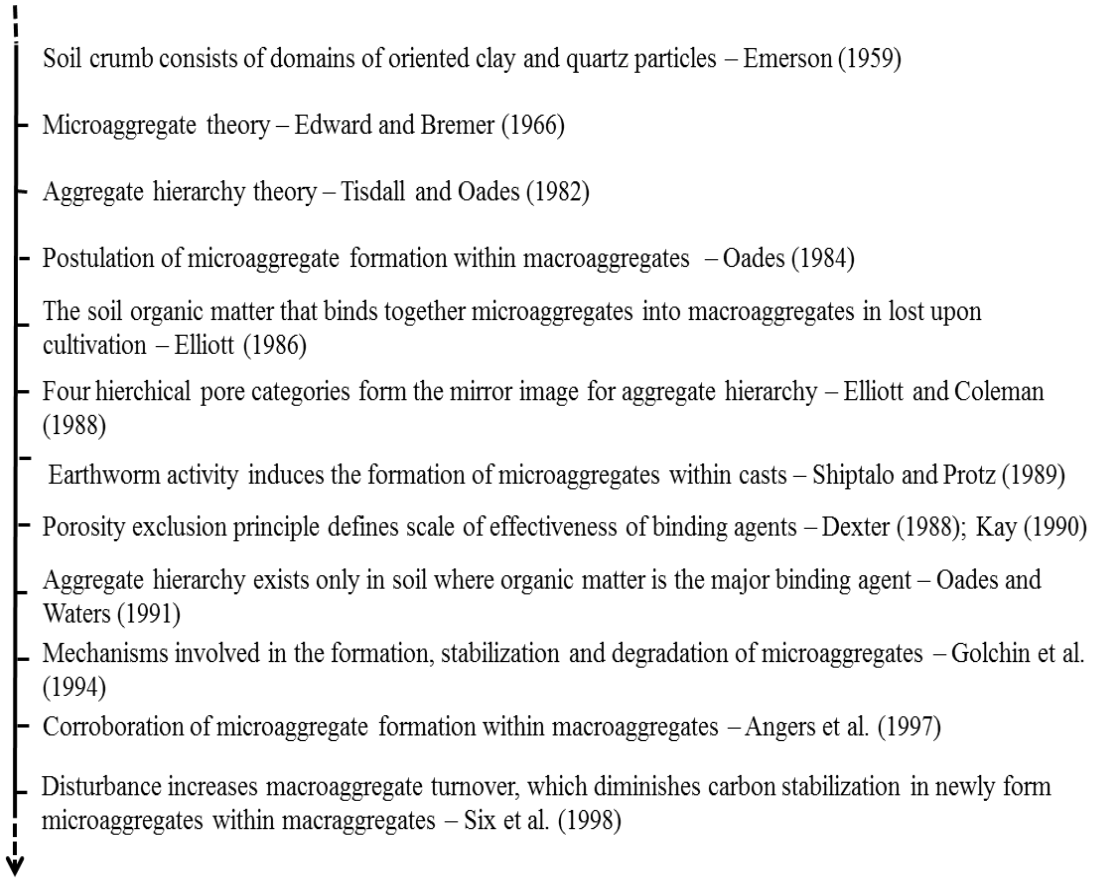


Figure 2.1. A time line of the critical advancements in the understanding of soil organic matter–aggregation interactions (Six et al., 2004).

In the aggregate hierarchy concept it is postulated that the different binding agents (i.e. transient versus temporary versus persistent binding agents) act at different hierarchical stages of aggregation. Free primary particles and silt-sized aggregates (<20 µm) are bound together into micro aggregates (20-250 µm) by persistent binding agents (i.e. humified OM and polyvalent metal cation complexes), oxides and highly disordered alumino-silicates. These stable microaggregates, in turn are bound together into macroaggregates (>250µm) by temporary (i.e. fungal hyphae and roots) and transient (i.e., microbial- and plant-derived polysaccharides) binding agents. These binding agents consist of plant roots, fungal hyphae and microbial or plant exudates (**Figure 2.2**).

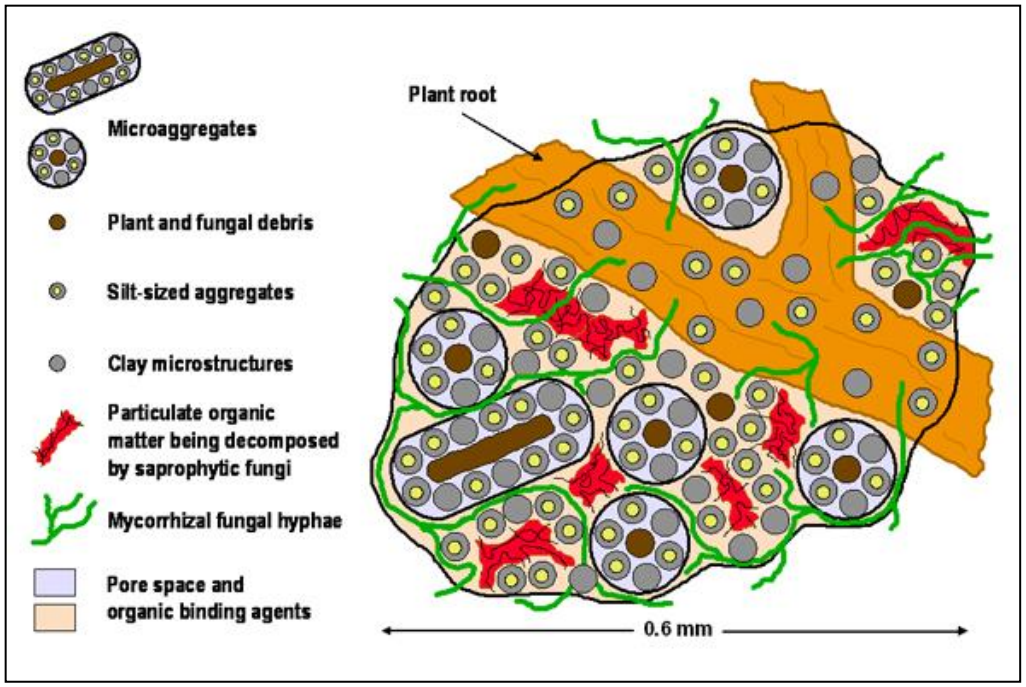


Figure 2.2. Conceptual diagram of soil aggregate hierarchy (Jastrow and Miller, 1998).

Elliott (1986) proposed that macroaggregates contain more labile and less highly processed SOM than microaggregates and that this SOM is lost upon cultivation. Oades and Waters (1991) concluded that aggregate hierarchy exists in soils where aggregate formation and stabilization are directed by organic matter but is not found in oxide-rich soils. Angers et al. (1997) postulated alternative models for aggregates formation with carbon. They presented quantitative data in corroboration of the concept of microaggregate formation within macroaggregates. Six et al. (1998) proposed a conceptual model to explain the SOM and aggregate dynamics in temperate soils (**Figure. 2.3**). The major concept of this model is that SOM is more stabilized in systems that are less subjected to physical disturbance (i.e. tillage, dry-wet and freeze-thaw cycles). This occurs due to the stabilization of greater amount of macroaggregates by protecting inter-microaggregate particulate organic matter lowering macroaggregate turnover, which then enhances the formation and stabilization of new microaggregates within macroaggregates and the accumulation of POM-C within these new

microaggregates. Disturbances such as tillage enhance macroaggregate turnover, which diminishes the formation of new microaggregates within macroaggregates and the protection of soil organic matter in these microaggregates (Six et al., 2000b).

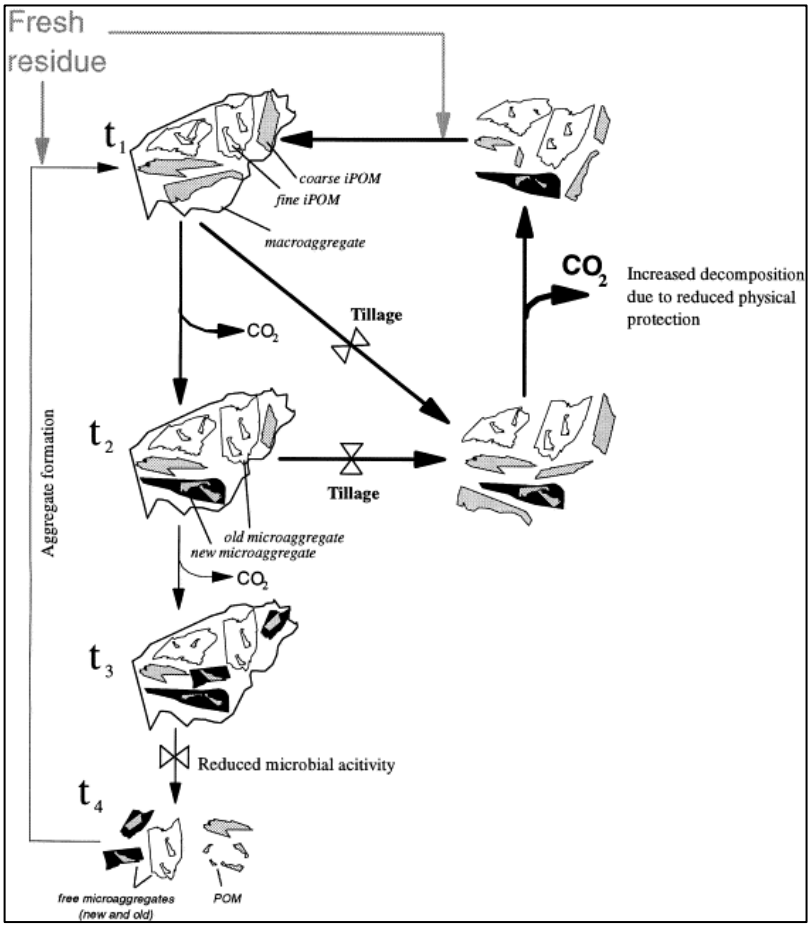


Figure 2.3. This conceptual model shows the "life cycle" of a macroaggregate and the formation of microaggregates (Six et al., 1998).

2.3.1. SOM distribution in aggregate fractions

Several studies have observed that macroaggregates often contain more OM than microaggregates, and this was suggested to be a result of macroaggregates including microaggregates plus OM serving as an intra-macroaggregate binding agent (Cambardella and Elliot, 1993; Jastrow et al., 1996; Puget et al., 2000; Six et al., 2000a).

Elliott (1986) suggested OM associated with macroaggregates was more labile than organic matter associated with microaggregates. Thus, POM is an especially labile pool of SOM (Balesdent et al., 1987), and plays an important role in the formation of macroaggregates (Jastrow and Miller, 1998; Gale et al., 2000; Puget et al., 2000; Six et al., 1998). The turnover times of occluded OM increase with decreasing aggregate size and various studies have shown that turnover times revealed by ^{13}C natural abundance were about 15-50 years for OM stored in macroaggregates (> 250 µm) and 100-300 years for OM in microaggregates (< 250 µm) (Angers and Giroux, 1996; Besnard et al., 1996; Johna et al., 2005; Monreal et al., 1997; Puget et al., 2000). However, very recently, AggModel estimated the turnover time of macroaggregates as 31 and 181 days for microaggregates (Segoil et al., 2013). The rapid turnover of macroaggregates reduces the formation of microaggregates within macroaggregates and the resulting stabilization of C within these microaggregates (Six et al., 1998, 1999, 2000b).

2.3.2. Factors affecting to the SOM dynamics of soil aggregates

There are various factors affecting the stabilization and distribution of SOM in aggregate fractions (**Table 2.4**). There are a number of studies reporting the moisture effect on the dynamics of SOM in aggregates and fewer focusing on the role of temperature (Plante et al., 2009). Land use and management practices were shown to significantly affected SOM contents of the aggregate fractions (Six and Paustian, 2014). Cultivation reduces SOM content and changes the distribution and causes a loss of C-rich macroaggregates and of POM fractions, in particular free POM, and results in an increase in C-depleted microaggregates (Besnard et al., 1996; Jastrow et al., 1996; Puget et al., 1995; Six et al., 2000b). Jagadamma and Lal (2010) showed that no-till systems resulted in significantly higher proportions of larger macroaggregates (> 2000 µm) increasing the soil organic C content by 1.5-2.8 times in all aggregate-size classes (>53, 53-250, 250-2000, >2000 µm). Consequently, microaggregate within macroaggregate C fractions may be a diagnostic fraction for changes in total SOC in response to changes in tillage management practice (Six and Paustian, 2013).

Table 2.4. Factors controlling the SOM dynamics in the aggregate fractions

Factor	**Parameters**	**References**
Climatic	Moisture	Bullock et al. (1988); Caron et al. (1992); Denef et al. (2001); Hayenes and Swift. (1990); Rasiah et al. (1992)
	Temperature	Plante et al. (2009)
Land use and management practices	Forest Pasture Cultivated 1. Tilled 2. Minimum tilled 3. Non-tilled	Angers et al. (1993); Balesdent et al. (2007); Bongiovanni and Lobartini (2006); Chung et al. (2008); Denef et al. (2004); Kong et al. (2005); Plante and McGill (2002); Six et al. (1999)
Soil	Plants – Types, roots and litter	Angers and Caron (1998); Degens (1997); Monreal et al. (1997); Puget et al. (2000)
	Macro-organisms	Bossuyt et al. (2004); Fonte et al. (2007, 2010); Johna et al. (2005); Jongmans et al. (2001); Pulleman et al. (2005a,b); Simpson et al., 2004; Yamashita et al. (2006)
	Micro-organisms	De Gryze et al. (2005)
	Binding agents	Bongiovanni and Lobartini, (2006); Abiven et al. (2007); Degens (1997); Simpson et al. (2004); Shipitalo and Protz (1989)
	Pore distribution	Dexter (1988); Elliott and Coleman (1998); Strong et al. (1998, 1999a,b,c)

In the early 20th Century, the research on SOM fractions, or pools, and soil aggregates was progressing separately, but as discussed, recent research has focused to link these together.

2.4. Soil carbon turnover models

SOM turnover models are being used increasingly in regional scale studies of soil organic carbon (SOC) dynamics (Falloon and Smith, 2002). The relationships between SOC and climate, site variables, and land use are important for modelling C cycling (Burke et al., 1989). SOC dynamics models can be divided into two categories depending on their internal structure (1) Process oriented (multi)-compartment models; (2) organism-oriented (food-web) models (Smith et al., 1998; Post et al., 2007). These models are based on conceptual SOM pools and have been shown to successfully simulate SOM dynamics over decadal time scales responding to organic matter inputs and environmental controls, such as temperature and moisture (**Table 2.5**).

Table 2.5. Main characteristics of processes-oriented versus organism-oriented models (Stockmann et al., 2013).

	Process-oriented models	**Organism-oriented models**
Model type	Mechanistic, predictive value	Mechanistic, explanatory value
Aim	Simulate processes involved in SOM migration and transformation	Simulate SOM using functional/taxonomic groups of the soil
Examples	CANDY (Franko, 1996), CENTURY (Parton, 1996), DAISY (Mueller et al., 1996), DNDC (Li et al., 1992), ITE (Thornley and Verbene, 1989), NCSOIL (Molina, 1996), Roth-C (Jenkinson and Coleman, 1994), Socrates (Grace et al., 2006), SOMM (Chertov and Komarov, 1996), Struc-C (Malamoud et al., 2009) and Verbene model (Verbene et al., 1990)	Fungal-growth models - Model of decomposition of OM that incorporate functional groups of microbial biomass (Paustian, 1985) Food web models based on taxonomic groups (mostly detrital models) (Hunt et al, 1987)
Representation of SOM	Different conceptual C pools with similar chemical or physical characteristics/differ by decomposition rates, stabilization mechanisms/generally soil biota only included in form of microbial biomass (exception: SOMM) Generally, more than one compartment of SOM degradation: (a) Active pool (fresh plant material, root exudates, microbial biomass) with MRT of 1 year (b) Slow pool (SOC that decomposes at intermediate rate) with MRT of 100 years	Microorganisms (bacteria, mycorrhizal and saprotrophic fungi) SOM and litter (represented in form of roots, detritus) SOC dynamics represented through different pools of soil biota (classified according to their taxonomy or metabolism) i.e. representation of soil biota by functional groups (food web models)

	Process-oriented models	**Organism-oriented models**
	(c) Passive or inert pool (SOC with physical or chemical stability) with MRT of 1000 years	
Mechanism	SOC decomposition based on first-order kinetic rates	C and N fluxes simulated through functional groups based on their specific death rates and consumption rates, applying energy conversion efficiencies and C:N ratios of the organisms
Time-step	Daily, weekly or monthly	Daily
Scale	Include top 30 cm of the soil	Small-plot
Application	Have been applied to a range of ecosystems (grassland, arable land, grass-arable rotations, forest)	Have been applied to arable land and grassland
Others	Successfully coupled with GIS software (e.g. CANDY, CENTURY, Roth-C)	Include changes of soil biota communities in the modelling of SOM dynamics (i.e. simulating feedback mechanisms due to changes in biota activity or characteristics)

2.5. Aggregate turnover models

SOM is the major stabilization agent and different types of aggregates. The turnover rate of the aggregates are detemined by type of SOM contributed to the aggregate formation. (Jastrow and Miller, 1998). A wide range of studies have been performed to describe the interactions between soil organic carbon (SOC) and soil structure. However, the impact of soil aggregate dynamics on SOM decomposition has not been explicitly incorporated in ecosystem models (Malamoud et al., 2009; Segoil et al., 2013).

To respond to this, Struc-C was proposed by Malamoud et al. (2009) which is a mathematical carbon model that describes how SOC influences the dynamics of soil structure, and consequently, soil physical behavior. In this model the smallest aggregates are composed of organo-mineral associations. The concept of this model is that organo-mineral associations are characterized by strong clay to SOC bonds, as the adsorption of SOC on clay is stronger than the bond among the soil organic compounds themselves. The clustering of these smaller aggregates forms larger aggregates. Three classes of aggregates are introduced in the model: Type1, Type2, and Type3 (**Figure 2.4**). Type1 corresponds to elementary organo-mineral associations. Type2 notionally may be regarded as microaggregates (63-250 μm) and Type3 is the largest and may notionally correspond to macroaggregates (> 250 μm) (Malamoud et al., 2009). For the first time a model was developed that correlated the amount of carbon present and the amount of aggregated material present. The model also was able to model the change in soil carbon and response in aggregate formation and depletion over time.

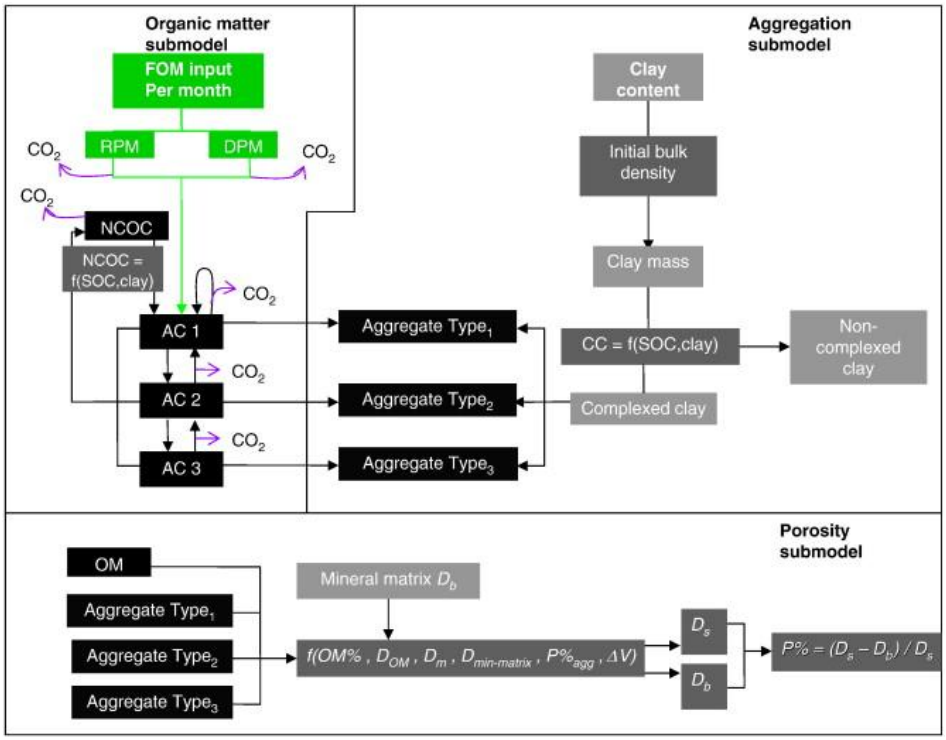


Figure 2.4. Schematic representation of the Struc-C model.

Recently, AggModel proposed by Segoli et al. (2013) which is based on the aggregate hierarchy concept (Oades, 1984; Tisdall and Oades, 1982) generally divides the soil mass into unaggregated (<53 μm), microaggregated (53–250 μm) and macroaggregated soil (>250 μm) (**Figure 2.5**). This hierarchical model creates four unique physical fractions: unaggregated soil external to macroaggregates (u), microaggregates external to macroaggregates (m), microaggregates within macroaggregates (mM) and non-microaggregated soil within macroaggregates (uM).

Macroaggregates (M, which is the sum of uM and mM) are formed by combining *u* and *m* fractions and macroaggregate breakdown transfers soil mass from the M back into the *u* and *m* fractions. Unlike Stuc-C, Aggmodel uses measured aggregate and carbon fractions to run the model, being four physical fractions (i.e. u, m, mM, and uM)

containing two organic matter fractions: particulate organic matter (POM) and mineral-associated organic matter (MAOM).

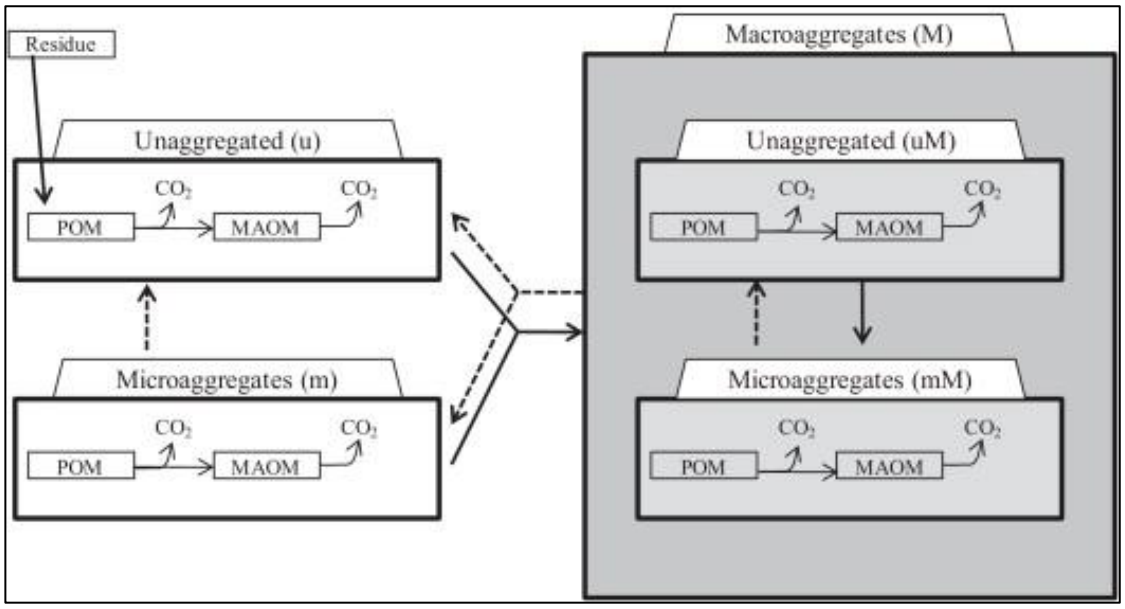


Figure 2.5. The conceptual model of AggModel (Segoli et al., 2013).

2.6. Quantification methods of SOC fractions

Quantification of different SOM pools is important for understanding C dynamics and their relative importance in the global C cycle (Trumbore, 1997). Instead of using conceptual SOM pools of SOM turnover models, there have been attempts to substitute these pools with measurable fractions of SOC (Skjemstad et al., 2004; Zimmermann et al., 2007b). Therefore, different fractionation procedures were proposed to separate SOM fractions from the bulk soils.

SOM fractionation methods are based on their pool sizes, chemical properties, and turnover rates. These fractionation procedures include physical and chemical separation methods and combinations of them. **Table 2.6** shows a summary of traditional chemical and physical fractionation methods and their limitations. Approximate time was

calculated considering excluding the soil and chemical preparation, drying and measuring of SOC. The calculated time was based on how much time require for separation using physical or chemical analytical steps described in the methodologies.

Physical fractionations procedures capture the effects of the spatial arrangement of primary and secondary organo-mineral particles on SOM dynamics (Olk and Gregorich, 2006). These procedures are based on the application of several disaggregating treatments (dry and wet sieving, slaking), dispersion (ultrasonic vibration in water) of increasing energy, followed by density separations and/or sedimentation (von Lützow et al., 2007). However, relatively large numbers of studies have failed to disperse soils completely by these proposed chemical and mechanical dispersion methods (Christensen, 1992). Alternately, while chemical fractionations are suitable for advanced chemical characterization and can be used to explain molecular-level interactions between SOM and nutrients or other organic compounds, these procedures cannot determine the spatial arrangement of the SOM (Olk and Gregorich, 2006). Chemical fractionation procedures involve the extraction of SOM in aqueous solutions, in organic solvents, on the hydrolysability of SOM with water or acids, and oxidation procedures. Each of the fractionation methods has provided useful information but all have procedural limitations (**Table 2.6**).

Table 2.6. Chemical and physical SOM fractionation procedures (von Lützow et al., 2007).

Method	**Chemical/ method used and Approximate time**	**Carbon fractions**	**Limitation**
Extraction	Water - 3 hours Chloroform - 3 days NaOH -30 minutes $Na_2P_2O_7$ - 1 hour K_2SO_4 - 2 hours	Dissolved organic matter Microbial biomass carbon Humic, fulvic and humin	These don't consider the spatial distribution on SOM in soils. thus, they do not distinguish physically inaccessible material (Inside aggregates or microspores)
Hydrolysis	Hot water- 1 day HCl/H_2SO_4 -1 day	Carbohydrates and protein celluloses, hemicelluloses lignin, cutin, suberin and waxes	Hot water hydrolysis is restricted to labile SOM and acid hydrolysis is not able to separate functional OM pools
Oxidation	$KMnO_4$ - 2 hours H_2O_2 - 2 hours $Na_2S_2O_8$ - 2 days	Labile C (sugar, organic acids, amino acids) Lignin, humic acid, alkyl C, clay+silty fraction	Removing only the active or passive pools.
Aggregate size fractionation	Dry sieving - 30 minutes Wet sieving – 3 hours Slaking – 3 hours	Free OM and occluded OM by microaggregates and macroagregates.	Aggregate fractionation have formed by different stabilization mechanisms and thus do not consist of the functional fractions needed for modelling

Method	**Chemical/ method used and Approximate time**	**Carbon fractions**	**Limitation**
Particle size fractionation	Dry sieving - 30 minutes Wet sieving - 3 hours	Active and passive pools	Not homogenous and in term s of turnover rate and cannot equivalent to model pools.
Density fractionation	Tetrabromoethane ($C_2H_2Br_4$) Bromoform ($CHBr_3$) Tetrachlromethane (CCl_4) Magnesium sulphate (Mg_2SO_4) Zinc bromide ($ZnBr_2$) Sodium iodide (NaI) Sodium polytungstate ($Na_6(H_2W_{12}O_4)$)	Light fraction and heavy fraction OM	Only makes a rough deffrenciation of active and passive pool.

2.7. Infrared spectroscopic techniques

Traditional soil analytical methods are slow, complicated and expensive, the links between the analytical data and relevant soil processes can be poorly understood (Janik et al., 1998). Consequence, there is a growing demand to improve the speed, number, quality of soil analysis to satisfy the demand for use in site-specific information, as well as to improve the utility of traditional approaches to routine soil testing (Janik et al., 1998). It is perhaps for these reasons that spectroscopic techniques are being considered as possible alternatives (or surrogates) to enhance or replace conventional laboratory methods of soil analysis (Viscarra-Rossel et al., 2006). Infrared spectroscopy is rapid, timely and cheap hence it is more efficient when a large number of analyses and samples are required with the conventional soil techniques (McCarty and Reeves, 2006). Infrared techniques are widely used to quantify the range of soil chemical, physical and biological properties as a simple and cost-effective method of analysis. However, very recently few studies were reported to use of these techniques to quantify the SOM pools

compared to traditional fractionation procedures (Skjemstad et al., 2004; Zimmermann et al., 2007b; Baldock et al., 2013).

Most of these techniques are are non-destructive and a single spectrum allows for simultaneous characterization of various soil constituents (Viscarra-Rossel et al., 2006). Particularly when used with multivariate analytical methods, simultaneous analyse many soil properties means that per analysis costs are low, with fast turnaround times. Digital output that can be directly applied using decision support software or as input to models. The quality of the analyses that are possible can only be as good as the quality of the data used for calibration and varies from near laboratory standard to indicator, "high, medium or low". The technique can also be used for classification or quality control purposes (Merry and Janik, 2001).

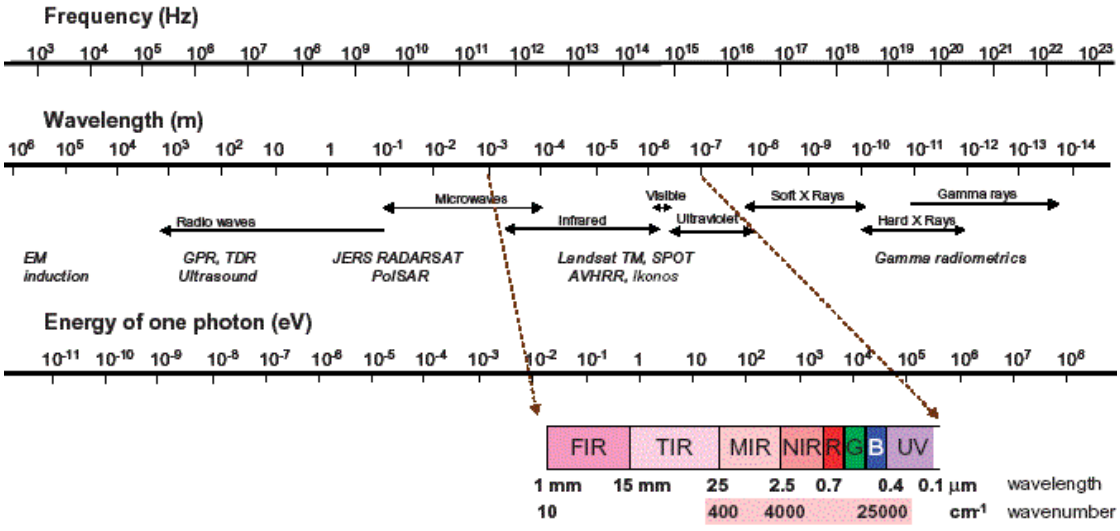


Figure 2.6. The electromagnetic spectrum (**Viscarra-Rossel et al., 2006**).

2.7.1. Near-infrared (NIR) spectroscopy

NIR utilizes electromagnetic spectra between the visible (400-700 nm) and infrared regions (700-2500 nm) (**Figure 2.6**) (Janik et al., 1998; McCarty and Reeves, 2000; Viscarra-Rossel et al., 2006). This has become popular for in field measurements of various soil properties, including SOM (Soriano-Disla et al., 2013). NIR is well supported commercially and can deal with larger bulk soil samples. because of its moreintense sources as it is well suited to field portability, remote sensing, copes better with moist samples and can deal with large number of samples. Portable instruments of NIR exist, such as the AgriSpec, ASD, Inc., Boulder (Reeves III, 2010), but commonly the process involves scanning soils that are air dried and sieved to < 2mm. There are few studies testing the effect of prediction performance of soil exposed to different grinding procedures. Fystro (2002) found a slightly increased prediction error for organic carbon and total nitrogen using air-dried, ball-milled (< 0.5 mm) soil compared with coarse (< 4 mm) soil. Recent studies found that external environmental conditions, such as temperature, soil moisture, and soil structural conditions are affecting to NIR reflectance (Minsany et al., 2011; Malley et al., 2000).

2.7.2. Mid infrared (MIR) spectroscopy

MIR (2500-25000 nm) spectroscopy (MIR) is an efficient method to predict various forms of carbon in the soil (Janik et al., 2007; Zimmermann et al., 2007b; Bornemann et al., 2008). MIR instruments are more expensive than visible -NIR and NIR measurements. Because, MIR transmitting materials and optical fibers are expensive and quite difficult to manipulate (Wilks, 2006). Absorption in mid-infrared spectroscopy corresponds to fundamental bands of molecular vibrations, whereas absorptions in NIR correspond to overtones and combinations of these fundamental bands (Williams and Norris, 1987). Therefore, MIR spectroscopy usually produces accurate predictions than NIR as it much more intense, and more spectral information is available. Since molecules differ from each other by having different combinations of functional groups, their MIR spectra can be used to identify them and characterize their structure.

Absorption bands associated with individual components in a mixture are frequently isolated from other bands and can be used to quantify the individual components by the strength of their absorption (Wilks, 2006).

Sample preparation is more important for MIR compared to NIR as relative adverse effect of sample heterogeneity on relatively smaller sampling area of the MIR beam. Thus, MIR spectra are generally obtained when samples are ground to < 100 μm and dried at 40°C (Stumpe and Weihermüller, 2011).

2.7.3. Spectra pre-processing

Usually soil spectra contain hundreds or thousands of reflectance values as a function of wavelength. As the first step of spectra pre-processing, to attempt linearization between absorbance and concentration, the measured reflectance (R) spectra is transformed in to log 1/R. Scattering effects can occur due to particle size distribution of the samples. Various scatter corrections have been used to enhance the more chemically relevant peaks in the spectra and reduce effects such as baseline shifts. For example, some of the more commonly used techniques include multiplicative scatter correction (MSC), first and second derivatives, simple additive baseline correction, the standard normal variate transform (SNV) with or without detrending and orthogonal signal correction (OSC) (Stanberg et al., 2010). Then, smoothing algorithms such as Savitzky–Golay transformation (Savitzky and Golay, 1964) is used to reduce noise in spectral signals. Recent studies have shown that smoothed and compressed soil spectra resulted in simpler and more robust calibrations (Viscarra-Rossel and Lark, 2009). These spectral processing steps are important to develop a good prediction model using chemometric regression models.

2.7.4. Modeling of NIR and MIR spectra

There are range of different multivariate techniques that are used to model the complex relationships with spectra signatures and soil properties. Many calibration methods including multiple regression analysis (MRA), principal component regression (PCR), partial least squares (PLS), neural boosted regression trees has mostly used to predict the soil attributes by spectra data and (Walvoort et al. 2006). Recently, Minasny and McBratney (2009) proposed the Cubist model was as an alternative in handling soil spectra. They have found cubist model gives high prediction accuracy, easy to interpret, and has automatic variable selection that makes it parsimonious.

It is an important requirement to select representative calibration and internal validation samples and an independent validation or "test" set when developing models for prediction of properties using spectroscopy techniques. The calibration samples should cover the variability expected in the full sample set and future unknowns (spectra and reference analytical data) (Soriano-Disla et al., 2013) and the validation (test) set must be independent of the calibration set in order to avoid an optimistic assessment of predictive performance. The quality of developed models can be evaluated as internal validation methods. Cross-validation generally gives an optimistic assessment of the actual performance of the models, particularly when the number of samples is small. With this method, each sample (leave one-out cross-validation), or group of samples, is removed in turn from the calibration set and its value is predicted as an unknown from models built from the remaining samples in the set (Dardenne et al., 2000) The splitting the data set at the beginning of the analysis as calibration dataset and validation dataset is a commonly used approach in data rich satiations. and using independent data collected in a different survey. This approach as the step is to set aside a randomly selected test set (normally 25% of the full data set). The next step is to derive, train, and optimize the model by either splitting into calibration and internal validation set (Soriano-Disla et al., 2013).

2.7.5. Evaluation of model performance

The quality of the developed calibration models and validation results are evaluating using different indices. The most commonly used indices are the coefficient of determination (R^2), root mean square error (RMSE)., the ratio to performance deviation (RPD), bias, Ratio of performance to inter-quartile distance (RPIQ) (**Table 2.7**)

Table 2.7. Model performances evaluation indices

Index	Description	Values
Coefficient of determination (R^2) (Williams, 1987).	Square of between the measured and their predicted values.	> 0.91 - Excellent 0.82 - 0.90 - Good 0.66 - 0.81 - Quantitative
Root mean squared error (RMSE)	The differences between value (Sample and population values) predicted by a model or an estimator and the values actually measured	Low RMSE higher accuracy
Ratio of performance to deviation (RPD) (Chang et al., 2001)	RPD = SD/RMSE (SD is Standard deviation)	> 2.0 - Excellent 1.4-2.0 - Fair < 1.4 - Non-reliable
Bias (Viscarra Rossel et al., 2008)	Measured mean minus the predicted mean	Low bias, higher accuracy
Ratio of performance to inter-quartile distance (RPIQ) Bellon-Maurel et al. (2010).	The difference between the 75th and 25th quartile of the validation data divided by the standard error of prediction	High RPIQ, higher accuracy

2.8. The need to reconcile carbon turnover and aggregate turnover model

The turnover of the soil aggregates directly control the stabilization and physical protection of SOM. Therefore, quantifying aggregate dynamics will improve the ability to predict SOM behaviour as affected by ecosystem management and global change. The existence of both Struc-C and Aggmodels are the first in an attempt to incorporate the physical protection of SOM in to ecosystem models.

However, a major limitation of all these models is that there are no direct and satisfactory methods to measure the amount of C contained in each of these pools within a soil sample (Six et al., 2002a; Christensen, 1996; Elliot et al., 1996). If these conceptual pools could be related to measurable fractions, it would be possible to initialize the model without the need to input historical data or run the model repeatedly assuming equilibrium conditions; it would also be possible to validate the model using the size of each pool as well as the total soil organic carbon (SOC). Consequently, Zimmermann et al. (2007a) and Skjemstad et al. (2004) attempted to substitute the conceptual pools in Roth-C model with measurable SOM fraction by using combined chemical and physical fractionation methods. In latter, some studies have investigated the use of MIR spectroscopy to predict these pools from fully dispersed soils (Janik et al., 2007; Zimmermann et al., 2007b; Baldock et al., 2013). There are few studies reported use of NIR and MIR techniques to quantify the aggregate related soil properties (Chang et al., 2001; Sarkhot et al., 2006; Madari et al., 2006). Therefore, development of integrated efficient quantification method to separate carbon fractions in both aggregate and carbon turnover model is crucial to perform in efficiently in ecosystem levels.

2.9. References

Abiven, S., Mensasseri, S., Angers, D.A., Leterme, P., 2007. Dynamics of aggregate stability and biological binding agents during decomposition of organic materials. European Journal of Soil Science. 58, 239-247.

Amundson, R., 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences. 29, 535-562.

Angers, D.A., Samson, N., Légère, A., 1993. Early changes in water-stable aggregation induced by rotation and tillage in a soil under barley production. Canadian Journal of Soil Science. 73, 51-59.

Angers, D.A., Giroux, M., 1996. Recently deposited organic matter in soil water-stable aggregates. Soil Science Society of America Journal. 60, 1547-1551.

Angers, D.A., Recous, S., Aita, C., 1997. Fate of C and nitrogen in water-stable aggregates during decomposition of $^{13}C^{15}N$ labeled wheat straw in situ. European Journal of Soil Science. 48, 295-300.

Angers, D.A., Caron, J., 1998. Plant-induced changes in soil structure: processes and feedbacks. Biogeochemistry. 45, 55-72.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry. 31, 697-710.

Baldock, J.A., Nelson, P.N., 2000. Soil organic matter. In: Summer, M., (Eds.), Handbook of Soil Science. CRC Press, Boca Raton, FL. pp25-84.

Baldock, J.A., 2007. Composition and cycling of organic carbon. In: Marschner, P., Rengel, Z., (Eds.), Soil Nutrient Cycling in Terrestrial Ecosystems. Springer Berlin, Heidelberg. pp1-35.

Baldock, J.A., Hawke, B., Sanderman, J., Macdonald, L.M., 2013. Predicting contents of carbon and its component fractions in Australian soils from diffuse reflectance mid-infrared spectra. Soil Research. 51, 577-595.

Balesdent, J., Mariotti, A., Guillet B., 1987. Natural ^{13}C abundance as a tracer for soil organic matter dynamics studies. Soil Biology and Biochemistry. 19, 25-30.

Besnard, E., Chenu, C., Balesdent, J., Puget, P., Arrouays, D., 1996. Fate of particulate organic matter in soil aggregates during cultivation. European Journal of Soil Science. 47, 495-503.

Bongiovanni, M.D., Lobartini, J.C., 2006. Particulate organic matter, carbohydrate, humic acid contents in soil macro- and microaggregates as affected by cultivation. Geoderma. 136, 660-665.

Bornemann, L., Welp, G., Brodowski, S., Rodionov, A., Amelung, W., 2008. Rapid assessment of black carbon in soil organic matter using mid-infrared spectroscopy. Organic Geochemistry. 39, 1537-1544.

Bornemann, L.C., Kookana, R.S., Welp, G., 2007. Differential sorption behaviour of aromatic hydrocarbons on charcoals prepared at different temperatures from grass and wood. Chemosphere. 67, 1033-1042

Bricklemyer, R.S., Miller, P.R., Paustian, K., Keck, T., Nielsen, G.A., Antle, J.M., 2005. Soil organic carbon variability and sampling optimization in Montana dryland wheat fields. Journal of Soil Water Conservation. 60, 42-51.

Brodowski, S., John, B., Flessa, H., Amelung, W., 2006. Aggregate-occluded black carbon in soil. European Journal of Soil Science. 57, 539-546.

Bullock, M.S., W.D. Kemper, W.D., Nelson, S.D., 1988. Soil cohesion as affected by freezing, water content, time and tillage. Soil Science Society of America Journal. 52, 770-776.

Bundt, M., Widmer, F., Pesaro, M., Zeyer, J., Blaser, P., 2001. Preferential flow paths: biological 'hot spots' in soils. Soil Biology and Biochemistry. 33, 729-738.

Buyanovsky, G.A., Aslam, M., Wagner, G.H., 1994. Carbon turnover in soil physical fractions. Soil Science Society of America Journal. 58, 1167-1173.

Caron, J., Kay, B.D., Stone, A., 1992. Improvement of structural stability of a clay loam with drying. Soil Science Society of America Journal. 56, 1583-1590.

Cambardella, C.A., Elliott, E.T., 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal. 57, 1071-1076.

Chabbi, A., Rumpel, C., 2009. Organic matter dynamics in agro-ecosystems-the knowledge gap. British Society of Soil Science, European Journal of Soil Science. 60, 153-157.

Chang, C.W., Laird, D., Mausbach, M.J., Hurburgh, C.R., 2001 Near-infrared reflectance spectroscopy-Principal components regression analyses of soil properties. Soil Science Society of America Journal. 65, 480-490.

Chertov, O.G., Komarov, A.S., 1996. SOMM - a model of soil organic matter and nitrogen dynamics in terrestrial ecosystems. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets. NATO ASI Series I. Springer-Verlag, Heidelberg, pp. 231-236.

Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science. 52, 345-353.

Chung, H., Grove, J.H., Six, J., 2008. Indications for soil C saturation in a temperate agroecosystem. Soil Science Society of America Journal. 72, 1132-1139.

Coleman, K., Jenkinson, D.S., 1996. RothC-26.3-a model for the turnover of carbon in soil. In: Powlson, D.S., Smith, P., Smith, J.U., (Eds.), Evaluation of Soil Organic Matter Models, NATO ASI Series, vol. I 38. Springer, Berlin. pp237-246.

Cremers**,** D**.**A**.**, Ebinger**,** M**.**H**.**, Breshears**,** D**.**D**.**, Unkefer**,** P**.**J**.**, Kammerdiener**,** S**.**A**.**, 2001. Measuring total soil carbon with laser-induced breakdown spectroscopy (LIBS). Journal of Environmental Quality. 30, 2202-2206**.**

Cremers, D.A., Radziemski, L.J., 2006. Handbook of Laser-Induced Breakdown Spectroscopy. Wiley, New York.

Dardenne, P., Sinnaeve, G., Baeten, V., 2000. Multivariate calibration and chemometrics for near infrared spectroscopy: Which method? Journal of Near Infrared Spectroscophy. 8, 229-237.

Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. Australian Journal of Soil Research. 35, 431-459.

De Gryze, S., Jassogne, L., Six, J., Bossuyt, H., Herman, S., Wevers, M., Merckx, R., 2006. Pore structure changes during decomposition of fresh residue: X-ray tomography analyses. Geoderma 134, 82-96.

Denef, K., Zotarelli, L., Boddey, R.M., Six, J., 2007. Microaggregate-associated C as a diagnostic fraction for management-induced changes in soil organic carbon in two Oxisols. Soil Biology and Biochemistry. 39, 1165-1172.

Denef, K., Six, J., Paustian, K., Merckx, R., 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry-wet cycles. Soil Biology and Biochemistry. 33, 2145-2153.

Derenne, S., Largeau, C., 2001. A review of some important families of refractory macromolecules: composition, origin, and fate in soils and sediments. Soil Science. 166, 833-847.

Dexter, A.R., 1988. Advances in characterization of soil structure. Soil and Tillage Research. 11, 199-238.

Edwards, A.P., Bremner, J.M., 1967. Microaggregates in soils. Journal of Soil Science. 18, 64-73.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils1. Soil Science Society of America Journal. 50, 627-633.

Emerson, W.W., 1959. Stability of Soil Crumbs. Nature. 183, 538-538.

Encyclopaedia Britannica., 1990. Humus. In: The New Encyclopaedia, Britannica, Micropaedia, Encyclopaedia Britannica, Inc. pp147.

Falloon, P.D., Smith, P., 2000. Modelling refractory soil organic matter. Biology and Fertility of Soils. 30, 388-398.

Feller, C., Blanchart, E., Bernoux, M., Lal, R., Manlay, R., 2012. Soil fertility concepts over the past two centuries: the importance attributed to soil organic matter in developed and developing countries. Archives of Agronomy and Soil Science. 58, S3-S21.

Fang, C.M., Smith, P., Moncrieff, J.B., Smith, J.U., 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature. 436, 881-881.

Franko, U., 1996. Modelling approaches of soil organic matter turnover within the CANDY system. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets. NATO Series I. Springer-Verlag, Heidelberg, pp. 247-254.

Fonte, S.J., Kong, A.Y.Y., van Kessel, C., Hendrix, P.F., Six, J., 2007. Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agro-ecosystem management. Soil Biology and Biochemistry. 39, 1014-1022.

Fonte, S.J., Barrios, E., Six, J., 2010. Earthworms, soil fertility and aggregate associated soil organic matter dynamics in the Quesungual agroforestry system. Geoderma. 155, 320-328.

Fystro, G., 2002. The prediction of C and N content and their potential mineralisation in heterogeneous soil samples using Vis-NIR spectroscopy and comparative methods. Plant and Soil. 246, 139-149.

Gale, W. J., Cambardella, C.A., 2000. Root-Derived Carbon and the Formation and Stabilization of Aggregates. Soil Science Society of America Journal. 64, 201-207.

Grace, P.R., Ladd, J.N., Robertson, G.P., Gage, S.H., 2006. SOCRATES - a simple model for predicting long-term changes in soil organic carbon in terrestrial ecosystems. Soil Biology Biochemistry. 38, 1172-1176.

Gregorich, E.G., Voroney, R.P., Kachanoski, R.G., 1991. Turnover of carbon through the microbial biomass in soils with different textures. Soil Biology and Biochemistry. 23, 799-805.

Hagedorn, F., Saurer, M., Blaser, P., 2004. A ^{13}C tracer study to identify the origin of dissolved organic carbon in forested mineral soils. Science. 55, 91-100.

Hassink, J., 1995. Decomposition rate constants of size and density fractions of soil organic matter. Soil Science Society of America Journal. 59, 1631-1635.

Haynes, R.J., Swift, R.S., 1990. Stability of soil aggregates in relation to organic constituents and soil water content. Journal of Soil Science. 41, 73-83.

Helfrich, M., Flessa, H., Mikutta, R., Dreves, A., Ludwig, B., 2007. Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. European Journal of Soil Science. 58, 1316-1329.

Herbert, B.E., Bertsch, P.M., 1995. Characterization of dissolved and colloidal organic matter in solutions: A review. In McFee, W.W., Kelly, J.M., (Eds.), Carbon Forms and Functions in Forest Soils, Soil Science Society of America, Madison, WI. pp62-88.

Islam, K.R., Weil, R.R. 2000. Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. Agriculture, Ecosystems and Environment. 79, 9-16.

Jagadamma, S., Lal, R., 2010. Integrating physical and chemical methods for isolating stable soil organic carbon. Geoderma. 158, 322-330.

Janik, L.J., Merry, R.H. Skjemstad, J.O., 1998. Can mid infrared diffuse reflectance analysis replace soil extractions?. Australian Journal of Experimental Agriculture. 38, 681-696.

Janik, L.J., Skjemstad, J.O., Sheherd, K.D., Spouncer, L.R., 2007. The prediction of soil carbon fractions using mid-infrared-partial least square analysis. Soil Research. 45, 73-81.

Janik, L.J., Forrester, S.T., Rawson, A., 2009. The prediction of soil chemical and physical properties from mid-infrared spectroscopy and combined partial least-squares regression and neural networks (PLS-NN) analysis. Chemometrics and Intelligent Laboratory Systems. 97, 179-188.

Jastrow, J.D., Miller, R.M., 1998. Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Soil Processes and the Carbon Cycle. CRC Press, Boca Raton, 207-223.

Jenkinson, D.S., Hart, P.B.S., Rayner, J.H., Parry, L.C., 1987. Modelling the turnover of organic matter in long-term experiments at Rothamsted. INTECOL Bull. 15, 1-8.

Jenkinson, D.S., Andrew, S.P.S., Lynch, J.M., Goss, M.J., Tinker, P.B. 1990. The turnover of organic carbon and nitrogen in soil. Philosophical Transactions: Biological Sciences. 329, 361-368.

Johna, B., Yamashitac, T., Ludwigb, B., Flessaa, H., 2005. Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma. 128, 63-79.

Jongmans, A.G., Pulleman, M.M., Marinissen, J.C.Y., 2001. Soil structure and earthworm activity in a marine silt loam under pasture versus arable land. Biology Fertility Soils. 33, 279-285.

Kaiser, E.A., Mueller, T., Joergensen, R.G., Isam, H., Heinemeyer, O., 1992. Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter. Soil Biology and Biochemistry. 24, 675-683.

Kay, B.D., 1990. Rates of change of soil structure under different cropping systems. Advances in Soil Science. 12, 1-52.

Kennedy, A.C., Papendick, R.I., 1995. Microbial characteristics of soil quality. Journal of Soil and Water Conservation. 50, 243-248.

Kleber, M., 2010. "What is recalcitrant soil organic matter?. Environmental Chemistry. 7, 320-332.

Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma. 113, 273–291.

Kong, A.Y., Six, J., Bryant, D.C., Denison, R.F., Van Kessel, C., 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Science Society of America Journal. 69, 1078-1085.

Krull, E.S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Functional Plant Biology. 30, 207-222.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science. 304, 1623-1627.

Lal, R., 2008. Carbon sequestration. Philosophical Transactions of the Royal Society: Biological Sciences. 363, 815-830.

Lawes, J., 1861. On the application of different manures to different crops and on their proper distribution on the farm. Private publication cited by Dyke (1993) (as per Heal et al., 1997).

Lawrence, C.R., Neff, J. C., Schimel, J. P., 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biology and Biochemistry. 41, 923-1934.

Li, C., Frolking, S., Frolking, T.A., 1992. A model of nitrous oxide evolution from soil driven by rainfall events. I Model structure and sensitivity. J. Geophysical Research. 97, 9759-9776.

Lohmann, R., McFarlane, J.K., Gschwend, P.M., 2005. Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York harbour sediments. Environmental Science and Technology. 39, 141-148.

Madari, B.E., Reeves, J.B., III, Machado, P.L.O.A., Guimaraes, C.M., Torres, E., McCarty, G.W., 2006. Mid- and near-infrared spectroscopic assessment of soil compositional parameters and structural indices in two Ferralsols. Geoderma. 136, 245-259.

Malamoud, K., McBratney, A.B., Minasny, B., Field, D.J., 2009. Modelling how carbon affects soil structure. Geoderma. 149, 19-26.

Malley, D.F., Martin, P.D., McClintock, L.M., Yesmin, L., Eilers, R.G., Haluschak, P., 2000. Feasibility of analysing archived Canadian prairie agricultural soils by near infrared reflectance spectroscopy. In ‘‘Near Infrared Spectroscopy: Proceedings of the 9th International Conference’’ In: Davies, A.M.C., Giangiacomo, R., (Eds.), NIR Publications, Chichester, UK. pp579-585.

Manlay, R.J., Feller, C., Swift, M.J., 2007. Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems and Environment. 119, 217-233.

Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: theory and models across scales. Soil Biology and Biochemistry. 41, 1355-1379.

McCarty, G.W., Reeves, J.B., Reeves, V.B., Follett, R.F., Kimble, J.M., 2002. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. Soil Science Society of America Journal. 66, 640-646.

McCarty, G.W., Reeves, J.B., 2006. Comparison of IR and MIR diffuse reflectance spectroscopy for field-scale measurement of soil fertility parameters. Soil Science. 171, 94-102.

McDowell, W.H., Likens G.E., 1988. Origin, composition, and flux of dissolved organic-carbon in the Hubbard brook valley. Ecological Monographs. 58, 177-195.

Minasny, B., McBratney, A.B., 2008. Regression rules as a tool for predicting soil properties from infrared reflectance spectroscopy. Chemometrics and Intelligent Laboratory Systems. 94, 72-79.

Minasny, B., Hartemink, A.E., 2011. Predicting soil properties in the tropics. Earth-Science Reviews. 106, 52-62.

Molina, J.A.E., 1996. Description of the model NCSOIL. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets. NATO ASI Series I. Springer-Verlag, Heidelberg, pp. 269-274.

Monreal, C.M., Schulten, H.R., Kodama, H., 1997. Age, turnover and molecular diversity of soil organic matter in aggregates of a Gleysol. Canadian Journal of Soil Science. 77, 379-388.

Mueller, T., Jensen, L.S., Hansen, S., Nielsen, N.E., 1996. Simulating soil carbon and nitrogen dynamics with the soil plant–atmosphere system model DAISY. In: Powlson, D., Smith, P., Smith, J.U., (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets. NATO ASI Series I, vol. 38. Springer-Verlag, Heidelberg. pp275-282.

Noon, C., Stevens, A., Oost, K.V., Six, J., Barthès, B., van Wesemael, B., 2014. Mid-infrared spectroscopy as a tool to identify and quantify soil organic carbon size fractions. Geophysical Research. 16.

Oades, J., Waters, A., 1991. Aggregate hierarchy in soils. Soil Research. 29, 815-828.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains Grasslands. Soil Science Society of America Journal. 51, 1173-1179.

Olk, D.C., Gregorich, E.G., 2006. Overview of the symposium proceedings, ‘‘meaningful pools in determining soil carbon and nitrogen dynamics’’. Soil Science Society of America Journal. 70, 967-974.

Parton, W.J., Ojima, D.S., Cole, C.V., Schimel, D.S., 1994. A general model for soil organic matter dynamics: sensitivity to litter chemistry, texture and management. In: R.B. Bryant, R.B., Arnoldm, R.W., (Eds.), Quantitative Modeling of Soil Forming Processes. Soil Science Society of America Journal. Madison, WI. pp147-167.

Paustian K, Schnürer J. 1987. Fungal growth response to carbon and nitrogen limitation: a theoretical model. *Soil Biology and Biochemistry*. 19, 613-620.

Piccolo, A., Mirabella, A., 1987. Molecular weight distribution of peat humic substances extracted with different inorganic and organic solutions. The Science of the Total Environment. 62, 39-46.

Pinstrup-Anderson, P., Hazell, P.B.R., 1985. The impact of the green revolution and prospects for the future. Food Reviews International. 1, 1-25.

Plante, A.F., McGill, W.B., 2002. Soil aggregate dynamics and the retention of organic matter in laboratory-incubated soil with differing simulated tillage frequencies. Soil Tillage Research. 66, 79-92.

Post, J., Krysanova, V., Suckow, F., Mirschel, W., Rogasik, J., Merbach, I., 2007. Integrated eco-hydrological modelling of soil organic matter dynamics for the assessment of environmental change impacts in meso- to macro-scale river basins. Ecological Modeling. 206, 93-109.

Puget, P., Chenu, C., Balesdent, J., 1995. Total and young organic matter distributions in aggregates of silty cultivated soils. European Journal of Soil Science. 46, 449-459.

Puget, P., Chenu, C., Balesdent, J., 2000. Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates. European Journal of Soil Science. 51, 595-605.

Pulleman, M.M., Six, J., Ujil, A., Marinissen, J.C.Y., Jongmans, A.G., 2005a. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. Applied Soil Ecology. 29, 1-15.

Pulleman, M.M., Six, J., Van Breemen, N., Jongmans, A.G., 2005b. Soil organic matter distribution and microaggregate characteristics as affected by agricultural management and earthworm activity. European Journal of Soil Science. 56, 453-467.

Rasiah, V., Kay, B.D., Marin, T., 1992. Variation of structural stability with water content: Influence of selected soil properties. Soil Science Society of America Journal. 56, 1604-1609.

Rice, C.W., 2006. Organic matter and nutrient dynamics. In Lal, R., (Ed.) Encyclopaedia of Soil Science. CRC Press, Boca Raton, FL. pp1180-1183.

Reeves, J.B., III, 2010. Near- versus mid-infrared diffuse reflectance spectroscopy for soil analysis emphasizing carbon and laboratory versus on-site analysis: Where are we and what needs to be done?. Geoderma. 158, 3-14.

Savitzky, A., Golay, M., 1964. Smoothing and differentiation of data by simplified least squares procedures. Analytical Chemistry. 36, 1627-1639.

Schmidt, M.W.I., Skjemstad, J.O., Gehrt, E., Kögel-Knabner, I., 1999. Charred organic carbon in German chernozemic soils. European Journal of Soil Science. 50, 351-365.

Segoli, M., De Gryze, S., Dou, F., Lee, L., Post, W.M., Denef, K., Six, J., 2013. AggModel: a soil organic matter model with measurable pools for use in incubation studies. Ecological Modelling. 263, 1-9.

Simpson, R.T., Frey, S.D., Six, J., Thiet, R.K., 2004. Preferential accumulation of microbial carbon in aggregate structures of no-tillage soils. Soil Science Society of America Journal. 68, 1249-1255.

Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal. 62, 1367-1377.

Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000a. Soil structure and organic matter distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal. 64, 681-689.

Six, J., Elliott, E.T., Paustian, K., 2000b. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry. 32, 2099-2103.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002a. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil. 241, 155-176.

Six, J., Jastrow, J.D., 2002b. Organic matter turnover. In: Lal, R., (Ed.), Encyclopedia of Soil Science. Marcel Dekker, Boca Raton, FL. pp936-942.

Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research. 79, 7-31.

Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. Soil Biology and Biochemistry. 68, A4-A9.

Skjemstad, J.O., Janik, L.J., Taylor, J.A., 1998. Non-living soil organic matter: what do we know about it? Australian Journal of Experimental Agriculture. 38, 667-680.

Skjemstad, J.O., Spouncer, L.R., Cowie, B., Swift, R.S., 2004. Calibration of the Rothamsted organic carbon turnover model (RothC ver. 26.3), using measurable soil organic carbon pools. Soil Research. 42, 79-88.

Smith, P., Smith, J.U., Powlson, D.S., McGill, W.B., Arah, J.R.M., Chertov, O.G., Coleman, K., Franko, U., Frolking, S., Jenkinson, D.S., Jensen, L.S., Kelly, R.H., Klein- Gunnewiek, H., Komarov, A.S., Li, C., Molina, J.A.E., Mueller, T.,

Parton, W.J., Thornley, J.H.M., Whitmore, A.P., 1997. A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. Geoderma. 81, 153-225.

Soriano-Disla, J.M., Janik, L.J. Viscarra-Rossel, R., Machdonald, L.M., McLaughlin, M.J., 2013. The Performance of visible, near-, and mid-infrared reflectance spectroscopy for prediction of soil physical, chemical, and biological properties. Applied Spectroscopy Reviews. 49, 139-186.

Strong, D.T., Sale, P.W.G., Heylar, K.R., 1998. The influence of the soil matrix on nitrogen mineralisation and nitrification. II. The pore system as a framework for mapping the organisation of the soil matrix. Australian Journal of Soil Research. 36, 855-872.

Strong, D.T., Sale, P.W.G., Heylar, K.R., 1999a. The influence of the soil matrix on nitrogen mineralisation and nitrification. III. Predictive utility of traditional variables and process location within pore system. Australian Journal of Soil Research 37, 137-149.

Strong, D.T., Sale, P.W.G., Heylar, K.R., 1999b. The influence of the soil matrix on nitrogen mineralisation and nitrification. IV. Texture. Australian Journal of Soil Research. 37, 329-344.

Strong, D.T., Sale, P.W.G., Heylar, K.R., 1999c. The influence of the soil matrix on nitrogen mineralisation and nitrification. V. Microporosity and manganese. Australian Journal of Soil Research. 37, 345-355.

Stvenson, F.J., Cole, M.A., 1999. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfer, Micronurients. Second Edition. John Wiley and Sons Inc. New York.

Stumpe, B., L. Weihermüller, L., Marschner, B., 2011. Sample preparation and selection for qualitative and quantitative analyses of soil organic carbon with mid-infrared reflectance spectroscopy. European Journal of Soil Science 62, 849-862.

Stockmann, U., Adams, M., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., de Courcelles, D., R. Singh, K., Wheeler, I., Abbott, L., Angers, D., Baldock, J., Bird, M., Brookes, P.C. , Chenu, C., Jastrow,J., Lal, R., Lehmann, C.J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M.,

The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agriculture, Ecosystems and Environment. 164, 80-99.

Thornley, J.H.M., Verbene, E.L.J., 1989. A model of nitrogen flows in grassland. Plant Cell Environment. 12, 863-886.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science. 33, 141-163.

Trumbore, S.E., Zheng, S.H., 1996. Comparison of fractionation methods for soil organic matter C-14 analysis. Radiocarbon. 38, 219-229.

Trumbore, S.E., 1997. Potential responses of soil organic carbon to environmental change. Proc. National Academy Science Letters. 94, 8284-8291.

Trumbore, S., 2009. Radiocarbon and soil carbon dynamics. Annual Rreview of Earth and Planetary Sciences. 37, 47-66.

van Veen, J.A., Ladd, J.N., Frissel, M.J., 1984. Modelling C and N turnover through the microbial biomass in soil. Plant and Soil. 76, 257-274.

Verbene, E.L.J., Hassink, J., De Willigen, P., Groot, J.J.R., Van Veen, J.A., 1990. Modelling soil organic matter dynamics in different soils. Netherland Journal of Agricultural Science. 38, 221-238.

Viscarra Rossel, R.A., Walvoort, D.J.J., McBratney, A.B., Janik, L.J., Skjemstad, J.O., 2006. Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. Geoderma. 131, 59-75.

Viscarra-Rossel, R.A., Lark, R.M., 2009. Improved analysis and modelling of soil diffuse reflectance spectra using wavelets. European Journal of Soil Science 60, 453-464.

von Lützow, M., Leifeld, J., Kainz, M., Kögel-Knabner, I. Munch, J.C., 2002. Indications for soil organic matter quality in soils under different management. Geoderma. 105, 243-258.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to

functional pools and to stabilization mechanisms. Soil Biology and Biochemistry. 39, 2183-2207.

Waksman, S.A., 1938. Humus. Origin, Chemical Composition and Importance in Nature, Second (Eds). The Williams and Wilkins Company, Baltimore, London, revised.

Wallerius, J.G., 1753. Mineralogie ou description generale des substances du regne animal (Mineralogy or General Description of Substances of the Animal Kingdom). Durand Pissot Ed, Paris.

Wander, M., Drinkwater, L.E., 2000. Fostering soil stewardship through soil quality assessment. Applied Soil Ecology. 15, 61-73.

Wander, M. 2004. Soil organic matter fractions and their relevance to soil function. In: Magdoff, F.a.R.R.W. (Ed.). Soil Organic Matter in Sustainable Agriculture. CRC Press LLC, Upper Saddle River, NJ.

Wilks, P., 2006. NIR vs Mid-IR - How to choose. Spectroscopy. 21, 43.

Zou, X. M., Ruan, H.H., Fu, Y., Yang, X.D., Sha, L.Q., 2005. Estimating soil labile organic carbon and potential turnover rates using a sequential fumigation-incubation procedure. Soil Biology and Biochemistry. 37, 1923-1928.

Yamashita, T., Flessa, H., John, B., Helfrich, M., Lud, B., 2006. Organic matter in density fractions of water-stable aggregates in silty soils: Effect of land use. Soil Biology and Biochemistry. 38, 222-3234.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J., 2007a. Measured soil organic matter fractions can be related to pools in the Roth-C model. European Journal of Soil Science. 58, 658-667.

Zimmermann, M., Leifeld, J., Fuhrer, J., 2007b. Quantifying soil organic carbon fractions by infrared-spectroscopy. Soil Biology and Biochemistry. 39, 224-231.

Zsolnay, A., 1996. Dissolved humus in soil waters. In: Piccolo, A. (Eds.), Humic Substances in Terrestrial Ecosystems. Elsevier, Amsterdam. pp171-224.

Chapter 3: Quantification of aggregated-carbon using Mid- and Near-infrared spectroscopic techniques

3.1. Introduction

Soil organic carbon is closely related to the formation and stabilisation of soil aggregates. According to the aggregate hierarchy model (Tisdall and Oades 1982) the smallest aggregates are composed of organo-mineral associations which are bound together with bacterial and fungal debris to form microaggregates (< 250 μm). The clustering of these microaggregates forms macroaggregates (> 250 μm) (**Figure 3.1a**). Oades and Waters (1991) determined that the aggregate hierarchy exists in soils where aggregate formation and stabilization are controlled by organic carbon. Further, Six et al. (2000) proposed that the organic carbon binding agents in macroaggregates degrade resulting in the release of the stable microaggregates contained. These microaggregates have the potential to form the building blocks for the next cycle of macroaggregate formation (**Figure 3.1b**). They also have shown macroaggregate turnover, microaggregate formation, and C stabilization within microaggregates is partly determined by C content of the soil.

Many studies have documented positive influences of aggregation on the accumulation of SOC (Six et al., 2002). Nearly 90% of SOC in surface soils was found to be located within aggregates and 20-40% of SOC as intra-microaggregate (Jastrow et al., 1996; Carter, 1996). It has been shown that inclusion of SOC in aggregates leads to a qualitative change and protects SOM from microbial decomposition (Six et al., 2002). As it is important to determine the distribution of SOC among the different aggregate fractions, physically separating soil into aggregate fractions is essential (Ashman et al., 2003). Aggregate fractionation to isolate free SOC and occluded organo-mineral associations is achieved using a combination of wet and dry sieving, and/or slaking methods. However, these fractionation procedures are costly and time-consuming to perform.

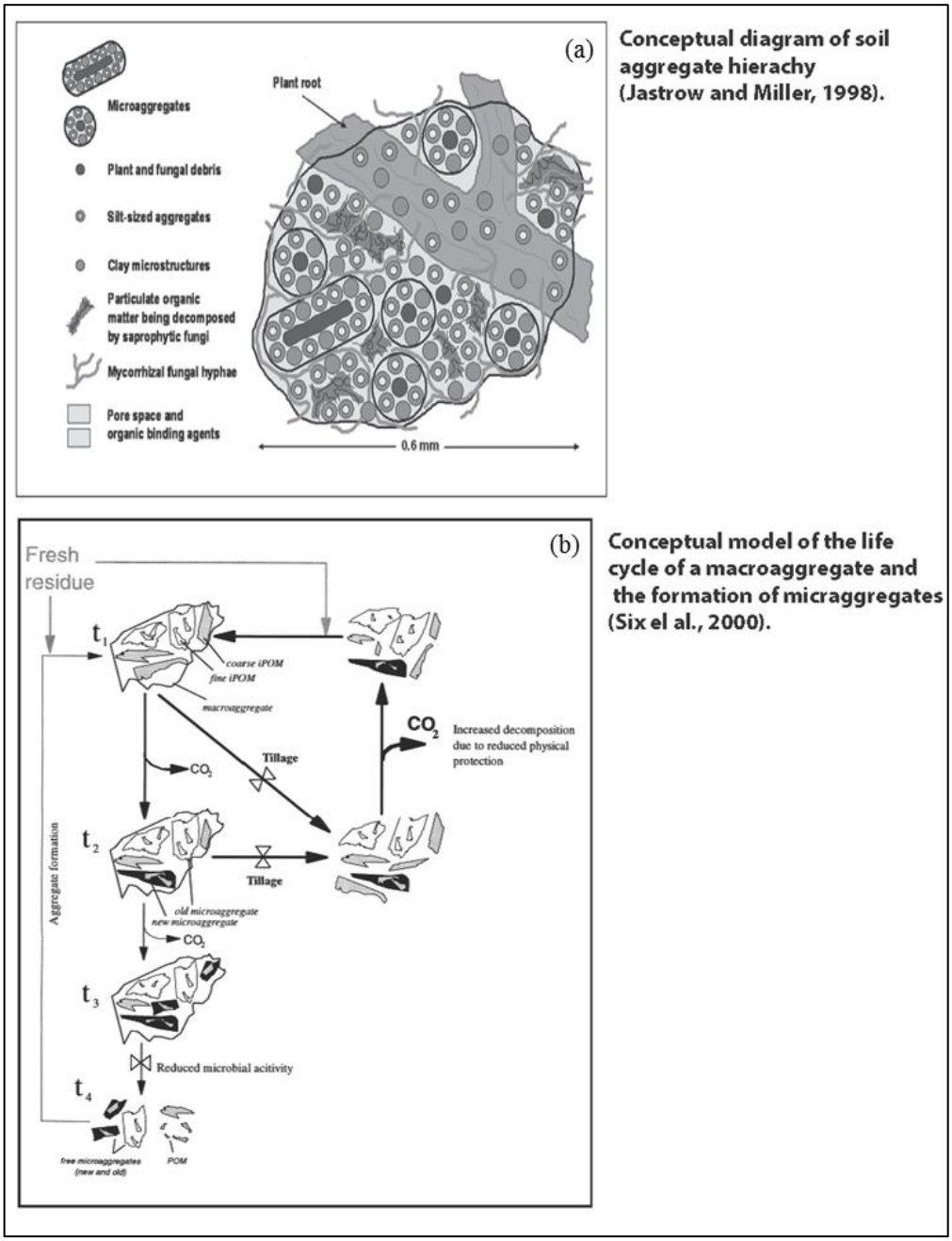


Figure 3.1. Conceptual model of (a) soil aggregate fractions and their components, and (b) the pathways affecting the formation and stabilization.

There is a growing demand for new soil analytical techniques to measure soil properties such as SOC that are faster and cheaper than traditional methods (McBratney et al., 2006). Spectroscopic methods have the potential to address this need by enabling samples to be scanned rapidly, inexpensively, and less destructive (Janik et al., 1998; Viscarra- Rossel et al., 2006). NIR uses electromagnetic radiation in the 400-2500 nm range and MIR spectroscopy radiation in the range from 2500-25000 nm. NIR spectroscopy has some advantages in terms of cost and portability of the instruments and potential to use as field instrument (Viscarra- Rossel et al., 2006). In contrast, peaks in the MIR are frequently better resolved, much more intense and more spectral information is available (McCarty et al., 2002).

There are large numbers of soil physical, chemical and biological properties that have been predicted from Vis-NIR, NIR and MIR spectra by using multivariate chemometric regression models (Soriano-Disla et al., 2013). However, only a few studies reported on prediction of soil structural properties such as soil aggregation by NIR and MIR spectra (Chang et al., 2001; Madari et al., 2006; Minasny et al., 2008; Sarkhot et al., 2007). Yet, no studies have been completed that assessed the efficacy of NIR and MIR spectra taken from whole soil to predict carbon in aggregate fractions which are isolated when studying aggregate hierarchies. To overcome the labour and costs of these separation techniques this research will investigate the efficiency of predicting soil carbon in aggregate fractions using bulk soil scanned by NIR and MIR.

3.2. Material and Methods

3.2.1. Soil sampling

Soil samples were obtained from a soil survey conducted in 2010 (Singh et al., 2011). This covered three major bio-regions of New South Wales, Australia, namely the South Eastern Highlands, NSW South Western Slopes and Brigalow Belt South (area of 158,000 km^2) (**Figure 3.2**). Mean annual rainfall of the studied area varied from 412-

987 mm. The land use types were cropping, grazing, modified pasture and grazing of natural vegetation. The sample sites were identified using Conditioned Latin Hypercube sampling from a regional scale soil survey (Minasny et al., 2006) using the covariates; predicted SOC (Wheeler et al., 2012), elevation, topographic wetness index, land use, gamma radiometrics region of interest of potassium (K, %), equivalent-thorium (eTh, ppm). Hundred and fifty soil samples were collected in this area to a depth of 30 cm. Forty five soil samples were selected from the survey based on TOC%, clay%, silt% and cation exchange capacity (CEC) to represent the maximum variability among the samples. These parameters where chosen as they are highly correlated with formation and stabilization of soil aggregates and therefore ensure that the sub-set of 45 samples maximize the potential variation present (Amézketa, 1999; Bronick and Lal, 2005; Six et al., 2002).

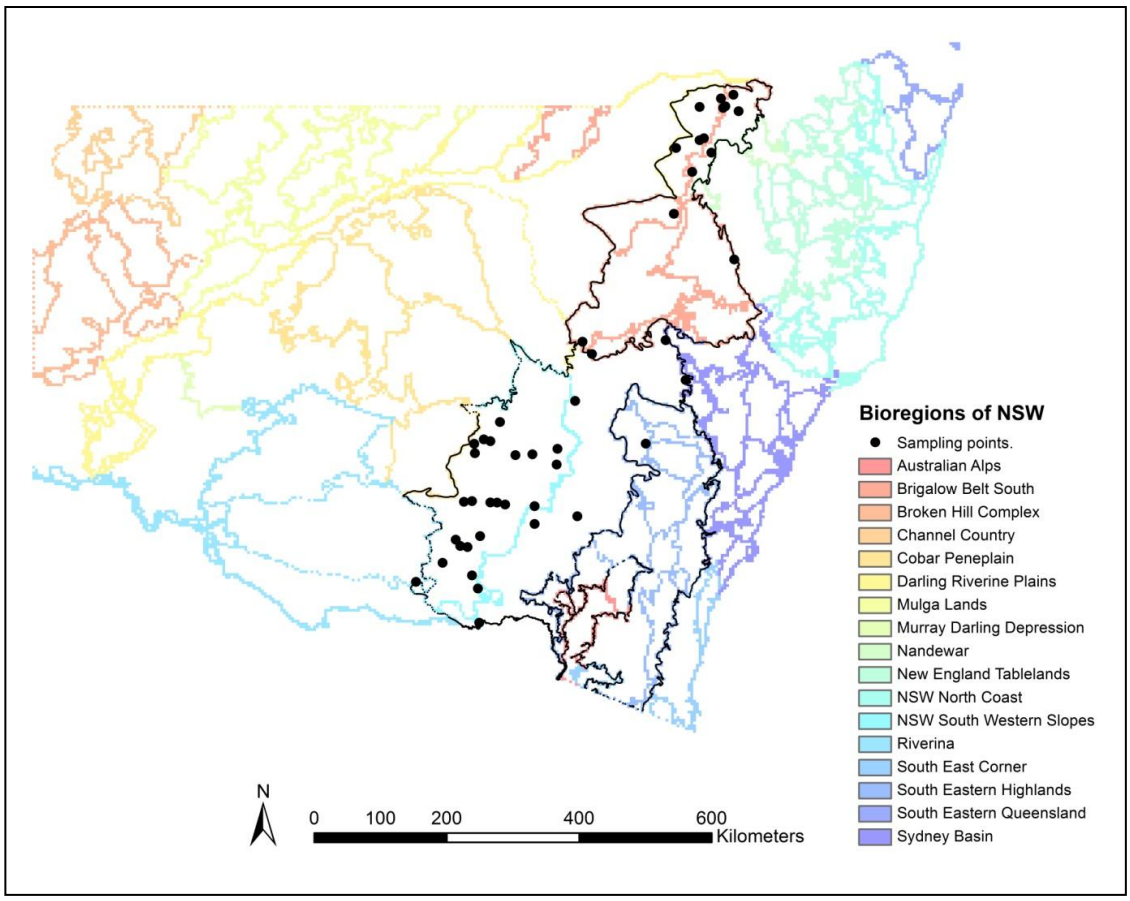


Figure 3.2. Study area and sampling sites.

3.2.2. Initial soil analysis

The soil samples included six soil orders, mainly chromosols, kandosols, kurosols, rudosols, sodosols and vertosols. Soil pH was measured at a ratio of 1:5 (w/w) of soil to water, using a pH meter (White, 1969). Soil texture was determined using hydrometer method (Gee and Bauder, 1986) on a 30 g subsample dispersed with 50 ml of 5% sodium-hexametaphosphate solution for 48 hours. TOC content was determined by an isotope ratio mass spectrometer (IRMS Delta V Thermo Finnigan, Bremen, and Germany).

The mean of pH, clay%, silt%, sand% and TOC% of the soil sub orders are represented in **Table 3.1.**

Table 3.1. Basic soil properties of the soil orders in the studied area

Soil order	pH	Clay (%)	Silt (%)	Sand (%)	TOC (%)
Chromosol	6.69	29.1	10.37	60.53	1.15
Kandosols	7.08	12.81	12.36	74.82	1.06
Kurosols	5.54	10.01	13.35	76.64	0.60
Rudosols	6.35	6.67	23.85	23.50	0.67
Sodosols	7.22	16.94	12.75	70.30	0.87
Vertosols	7.09	33.27	11.97	54.75	0.89

3.2.3. Aggregate separation

Soil aggregate separation was done using the wet-sieving method described by Six et al. (2000) illustrated in **Figure 3.3**. Hundred gram of air-dried soil was submerged for 5 minutes on a 2000 μm sieve. Aggregates were separated by moving the sieve (by hand) up and down 3 cm with 50 repetitions within 2 minutes. The > 2000 μm aggregates were collected and sieving was repeated for the < 2000 μm fraction with the 250 μm and 63 μm sieves. All aggregate fractions were air dried at 40 ^{0}C. Thus, three aggregate-size fractions were recovered: macroaggregates (250-2000 μm), microaggregates (63-250 μm) and silt- and clay-sized aggregates (< 63 μm) (Tisdall and Oades, 1982). The collected fractions were oven dried at 40°C and weighed. TOC of oven-dried aggregate fractions were determined by an isotope ratio mass spectrometer (IRMS Delta V Thermo Finnigan, Bremen, and Germany).

[3.1]

Figure 3.3. Wet sieving scheme used to obtained aggregate fractions and use of spectroscopic data.

Mass recoveries were calculated and obtained 98% mass recovery for soils as described Sanderman el al., 2011. (**Equation 3.1**)

$$\text{Mass Reccoveries } \% = \frac{\text{Dry mass of Aggregate fraction}}{\text{Dry mass of Total soil}} \times 100$$

Fractional carbon recoveries were maintained between 85-115% after the wet sieving (**Equation 3.2**).

$$\text{Carbon recovery } \% = \frac{\text{Carbon } \%\ \text{Aggregate (MAC + MIC + OMA)}}{\text{Carbon \% of Bulk soil}} \times 100 \quad [3.2]$$

Where, MAC is macroaggregates, MIC is microaggregates, OMA is organo-mineral associations.

3.2.4. Mid-Near infrared diffuse reflectance spectroscopy

Spectra were recorded using a Bruker Tensor 37 spectrophotometer equipped with an automated high throughput device (Bruker HTS-XT GmbH, Ettlingen, Germany) with OPUS software version 6.5. This is operating with a liquid N_2 cooled mercury-cadmium telluride (MCT) detector. Bulk soils were ground into 100 µm and about 20 mg bulk sample was transferred to micro plates. Soils were compacted to leave a plain and dense surface for measurement of the Diffuse Reflectance Infrared Fourier Transform (DRIFT). The spectra were collected at in a single run at a resolution of 4 cm^{-1} from 800

- 2500 nm (4000- 124500 cm^{-1}) with NIR and from 2500-25000 nm (600-4000 cm^{-1}) with MIR detector. KBr powder was used as the background and 60 scans were done for every sample to minimize errors in spectroscopic measurement.

3.2.5. Visible-Near-infrared spectroscopy

Vis-NIR spectra were collected by AgriSpec spectrometer with a contact probe (Analytical Spectral Devices, Inc., Boulder, Colorado, USA). The AgriSpec composes three detectors which provide a spectral range of 350-2500 nm. Air dried samples (< 2 µm) were illuminated by a halogen lamp and reflected light was transmitted to the spectrometer. A Spectralon (Labsphere, North Sutton, NH, USA) white reference was scanned as a reference spectrum. Each spectrum was taken by means of 30 internal scans.

3.2.6. Spectral analyses

MIR and NIR spectra taken from Bruker Tensor 37 spectrophotometer were reflectance spectra. The reflectance spectra collected from the Vis-NIR spectrometer were converted to absorbance spectra using the below relationship.

$$\text{Absorbance} = \log \frac{1}{\text{Reflectance}} \qquad [3.3]$$

The spectral range 500-2450 nm of Vis-NIR was used to exclude any increase in noise at the limits of the range (Minasny et al., 2011). The spectra were then reprocessed to a resolution of 2 nm. The best fitting derivative were performed correct for baseline difference between spectra and weak spectral signals. The spectra were smoothed using the Savitsky-Golay algorithm with a window size of 11 and a 2nd order polynomial (Savitzky and Golay, 1964).

3.2.7. Cubist regression model

The Cubist model (Kuhn et al., 2013) was used to calibrate the spectra against the measured TC content. Cubist was introduced as an alternative method in handling soil spectral data by Minasny and McBratney (2009). Cubist is a data-mining technique which consists of a set of comprehensible rules, where each rule has an associated multivariate linear model. Whenever a situation matches a rule's conditions, the associated model is used to calculate the predicted value (Minasny and McBratney, 2009). Cubist was shown to result in high accuracy, easy interpretation, variable selection, parsimony and respects the upper and lower boundary values of the predicant (Minasny et al., 2013).

The calibration models were developed using 45 bulk soil spectra. The models quality were performed by using leave-one out cross-validation (LOOCV). LOOCV carried out on a dataset created from a limited number of fields, leads to over-optimistic performance (Bellon-Maurel and McBratney, 2011). Each observation was removed from the data set and measures bulk and aggregated-C was predicted using remaining observations.

3.2.8. Model evaluations

The accuracy of the predictions was assessed using coefficient of determination (R^2), root mean squared error (RMSE), standard error of prediction (SEP), ratio of performance to deviation (RPD), and the ratio of the interquartile distance of the validation set to the standard error of prediction (RPIQ) (Bellon-Maurel et al., 2010).

$$\mathrm{RMSE\ (SEP)} = \sum_{i=1}^{m} \frac{\left(\hat{\mathrm{Y}}\mathrm{i} - \mathrm{Yi}\right)^2}{\mathrm{N}} \qquad [3.4]$$

$$\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{SEP}} \quad [3.5]$$

$$\mathrm{RPIQ} = (\mathrm{IQ}/\mathrm{SEP}) \quad [3.6]$$

where, Y is observed value; $\hat{Y}$ is predicted value; SD is standard deviation; IQ is interquartile distance of the validation set (IQ=Q3-Q1) and N is number of samples.

R^2 indicates how close the measures and predicted data compared and RMSE is related to the accuracy of the predictions. As general quality parameters, higher R^2, RPD, and RPIQ values with lower RMSE values were considered as good predictions of the models.

3.2.9. Mean differences of absorption spectra

Means absorptions were calculated for bulk soil, macroaggregates, microaggregates and organo-mineral associations' spectra. Absorption differences for aggregate fractions were calculated by the equation below.

$$\text{Absorption difference} = \text{Mean absorptions of BS} - \text{Mean absorptions of AGF} \quad [3.7]$$

Where, BS is Bulk soil spectra; AGF is aggregate fraction spectra.

3.3. Results and Discussion

3.3.1. Basic soil properties and carbon distribution of aggregate fractions

Table 3.2. shows mean values of TOC, pH and particle size distribution for the 45 bulk soil samples. The sampling protocol used has resulted in 45 samples with a large range in clay and silt contents, where the clay % ranges from 4 to 40% and as reported by Six

et al. (2004) is a key component of the aggregate formation and carbon stabilization of soil. The TOC of the soils varied from 0.25 - 2.98%. In general, organic carbon stocks in Australian soils are much lower than the global average. This study obtained on average 99.98% carbon recovery after wet sieving of the soils which ensured the accuracy of the aggregate seperationg using wet sieving method.

Table 3.2. TOC, pH and particles size distribution and mass recoveries and carbon recoveries of bulk soil.

Character	Mean	Sta. Dev.	Maximum	Minimum
pH	7.02	1.08	7.51	5.09
Clay (%)	23.03	8.68	43.3	4.55
Silt (%)	11.26	4.59	24.1	3.33
Sand (%)	65.01	9.33	86.71	49.65
TOC (%)	0.98	0.53	2.98	0.25
Mass recovery (%)	97.23	3.27	101.93	88.48
Carbon recovery (%)	99.98	10.84	118.84	74.84

Many studies have shown that soil carbon concentrations have a positively skewed distributions (Minasny et al., 2013) and the findings here are of no exception. Macroaggregate-C shows a highly positive skewness compared to the microaggregate-C and organo-mineral asociated-C. The relative C concentration for each aggregate fraction observed follows organo-mineral associations > macroaggregates > microaggregates (**Figure 3.4**) and this agrees with the work of Sarkhot et al. (2007) who observed for both wet-sieved and dry- sieved soil aggregates for particle size C greater in the 53 μm, followed by the 150 -250 μm, and then the 250 -2000 μm. Forty two

percent of the C was found in the organo-mineral associations which are characterized by strong clay to SOC bonds. The adsorption of SOC on clay is stronger than the bond between the soil organic compounds themselves (Christenson, 2001) and implies that this fraction will prevent SOC degradation compared to the SOC between microaggregates and microaggregates. Macroaggregates revealed higher C contentsthan microaggregates. However, this difference is not significant when comparing with several studies reporting greater concentration of C in macroaggregates compared to microaggregates (Cambardella and Elliot, 1993; Jastrow et al., 1996; Puget et al., 1995; Six et al., 2000). The macroaggregates often containe more SOC than microaggregates, because macroaggregates include microaggregates plus SOC serving as an intra-macro aggregate binding agents. Further, macroaggregates are associated with larger concentrations of soil mineralizable organic C, nutrients and microbial biomass meaning macroaggregates are enriched biologically (Oades, 1993).

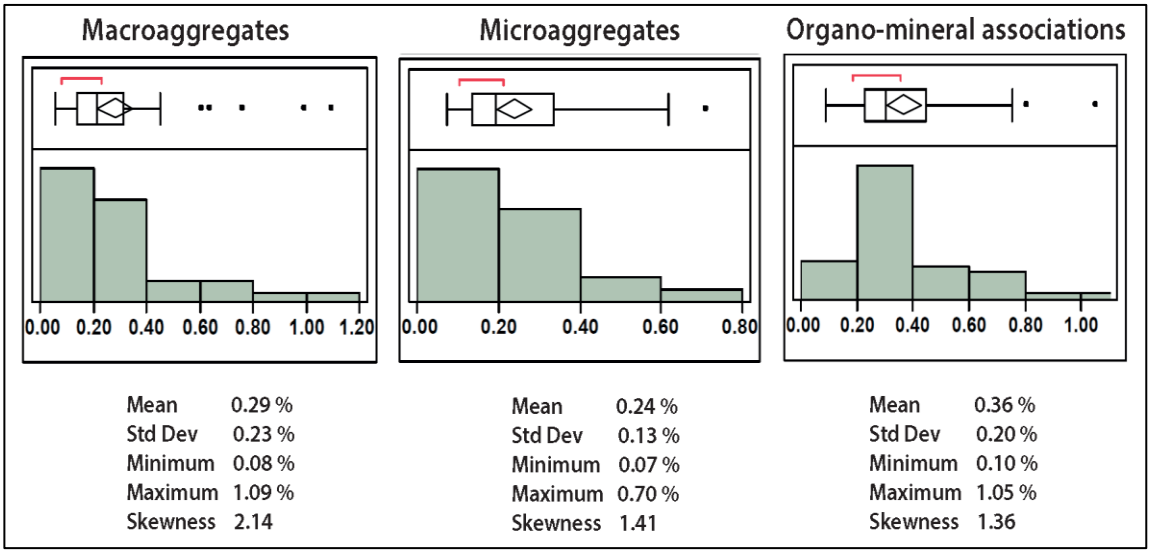


Figure 3.4. Histograms, outlier box-plots and statistical description of carbon (%) distribution of macroaggregates, microaggregates and organo-mineral associations.

3.3.2. Quantification of bulk soil TOC

Figure 3.5. shows the cross-validation results using measured and predicted TOC of bulk soil samples. There were no derivatives required to get the best fitting model by the MIR. However, first derivative was used for NIR and Vis-NIR to enhance the weak signals. Vis-NIR, NIR and MIR successfully quantified the bulk soil TOC. The MIR calibration resulted in the best prediction of TOC compared to NIR and Vis-NIR. The R^2 value of 0.86 with the lowest RMSE indicated better results for MIR than for NIR and Vis-NIR with R^2 values of 0.83 and 0.82 with RMSE value 0.22% and 0.21% respectively. The better validation results were obtained by MIR compared to NIR. According to the literature, MIR predictions of SOC have been reported to perform better than NIR and Vis-NIR (Soriano-Disla et al, 2014), as MIR is slightly superior to NIR with prediction errors (RMSE) always lower than the ones obtained with NIR(Bellon-Maurel and McBratney, 2011) Comparatively, the MIR peaks are frequently better resolved and much more intense and more spectral information is available for MIR (Reeves et al., 2001).

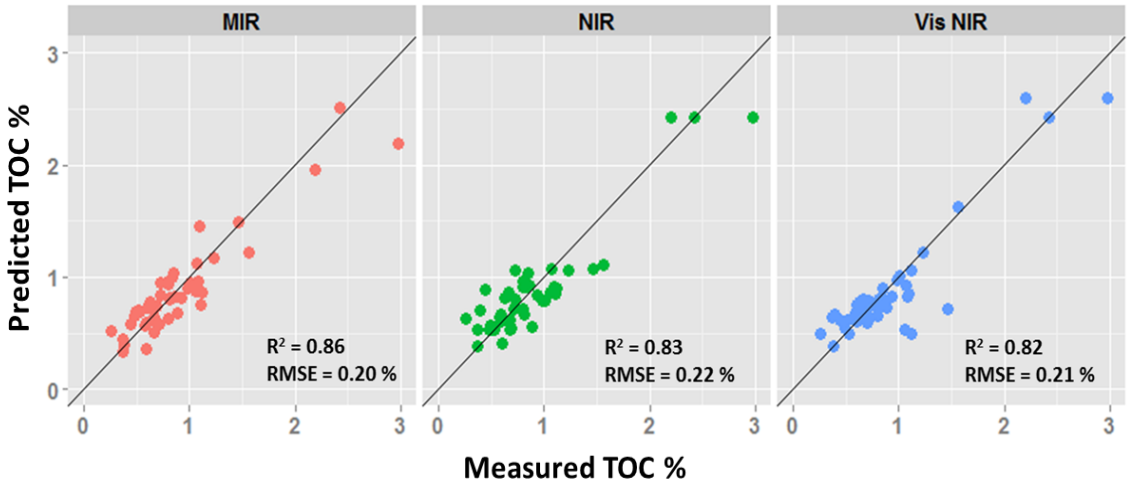


Figure 3.5. Cross-validation scatter plots of measured and Cubist predicted TOC % by MIR, NIR and Vis-NIR spectra.

3.3.3. Quantification of C in aggregate fractions

In this study physically fractionated aggregate C was predicted using MIR, NIR and Vis-NIR spectroscopy and validation models were assessed by using R^2 values, RMSE, RPD and RPIQ (**Table 3.3**). R^2 values give the degree of relationship which exists between measured and predicted C %. Williams (2003), has suggested R^2 value values of 0.91 or greater indicate 'excellent prediction', values between 0.82-0.90 'good', whereas values between 0.66-0.81 indicate an approximate 'quantitative' prediction. Thus, according to the results, macroaggregate-C and microaggregate-C by MIR and NIR and organo-mineral associated-C by MIR was reasonably predicted. In addition to the R^2 values the RMSE values were low (0.07-0.19%) in all the fractions revealing the accuracy of the models. The microaggregates fraction had a low RMSE % (0.07-0.11%) when compare to the other two aggregate fractions. RMSE is a good index to compare calibration model validations using the same validation sample set, depending on the measurement range of the data (BellonMaurel et al., 2010). The C content of microaggregates ranged from 0.07-0.70 % and slightly is lower than the other two fractions (**Figure 3.4**). C in macroaggregates showed the best R^2 value (0.85) with a RMSE of 0.17%. Vis-NIR prediction of organo-mineral associations was the least well predicted fractions of the results obtained.

RPD is a popular statistical parameter used for many years to evaluate the accuracy of calibration and validation models (Chang et al., 2001; Williams, 1987). According to the Cheng et al. (2001), macroaggregate C prediction was "excellent" by MIR and NIR spectra and all other aggregated-C fractions predictions were 'fairly well' from MIR, NIR and Vis-NIR. Howver, more recently, Minasny and McBratney (2013) have shown that both RPD and R^2 are the same measurements and that it would not be satisfactory to use the RPD classification to justify the perdition models. We also observed the same pattern between R^2 and RPD values of cross-validation results for both bulk and aggregate fractions (**Figure 3.5. and Table 3.3.**). These showed highest R^2 (0.87) and highest RPD (2.99%) by MIR in macroaggregate-C whereas, lowest R^2 (0.50) with

lowest RPD (1.57). According to Bellon-Maurel et al. (2010), RPD shows an artificially good performance for lognormal distributions and since soil carbon concentration distributions were highly skewed (lognormal or square root normal distributions) they proposed RPIQ as a more reliable index to evaluate spectroscopic validation results. Since the C distributions of aggregate fractions were lognormal (**Figure 3.4),** RPIQ has been used and showed highest values (0.93) for macroaggregated-C as predicated by MIR spectra and lowest in microaggregateC predicted by Vis-NIR.

Table 3.3. Cubist cross-validation statistics of C by using MIR, NIR and Vis-NIR spectra in aggregate fractions.

	Derivatives	**RMSE**	**R^2**	**RPD**	**RPIQ**
Macroaggregates					
MIR	None	0.17	0.85	2.99	0.93
NIR	First	0.14	0.77	2.20	0.69
Vis-NIR	First	0.19	0.57	1.65	0.51
Microaggregates					
MIR	First	0.09	0.64	1.78	0.51
NIR	None	0.11	0.66	1.79	0.53
Vis-NIR	First	0.09	0.55	1.57	0.45
Organo-mineral associations					
MIR	First	0.16	0.63	1.80	0.72
NIR	None	0.12	0.52	1.77	0.71
Vis-NIR	First	0.14	0.52	1.57	0.63

3.3.4. Qualitative analysis of MIR, NIR and Vis-NIR spectra

3.3.4.1. Use of Cubist model predated wavelengths

Figure 3.6, **3.7 and 3.8** shows the important variables used as predictor in the Cubist model by MIR, NIR and Vis-NIR spectra when predicting the C content of the aggregate fractions. Cubist provides the percentage of the variables used in model conditions and the wavelengths used as a predictor in the regression. Vertical bars represent the relative importance of particular wavelengths in predicting aggregated-C. The blue bar represents the wavelengths used in conditions, and the purple lines refer to wavelengths used in the regression model. **Table 3.4, 3.5** and shows a summary of important wavelengths and corresponding functional groups in all aggregate fractions obtained by MIR, NIR and Vis-NIR spectra. All MIR, NIR and Vis-NIR bulk soil spectra have used similar spectral bands to obtain the Cubist rule conditions and regression. The macroaggregate C was predicted mainly from the aliphatic regions from both MIR, NIR and Vis-NIR spectra (**Chapter 4** discusses the C pools related to these aggregate fractions). Iron, aluminum and polysaccharide bands were also used as predictors in the regression model. The Cubist model has not used any specific wavelength for its conditions for prediction of microaggregate C by MIR spectra. It has used wavelengths 14306 nm, 12626 nm, 9416 nm, 5232 nm, 4982 nm, 3756 nm and 2631 nm as predictors in the regression. Both NIR and Vis-NIR prediction were based on wavelengths mainly correspondent to aromatic compounds, carbohydrates and clay minerals. Spectral features related to silicates and clay minerals have shown large influence to predict the C % of organo-mineral associations.

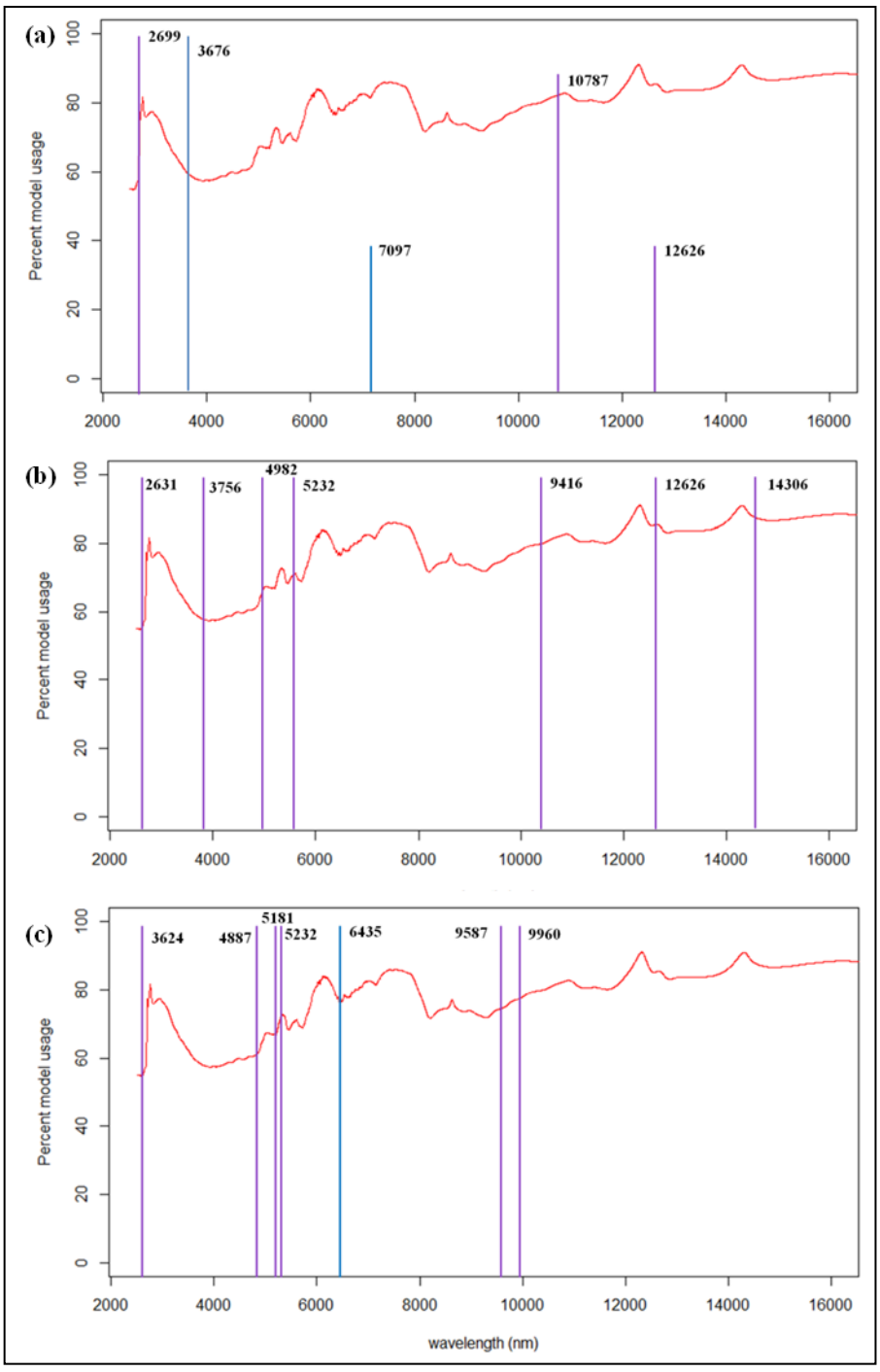


Figure 3.6. Important variables used in the Cubist model by bulk soil MIR spectra to predict carbon in macroaggregates (a), microaggregates (b) and organo-mineral associations (c).

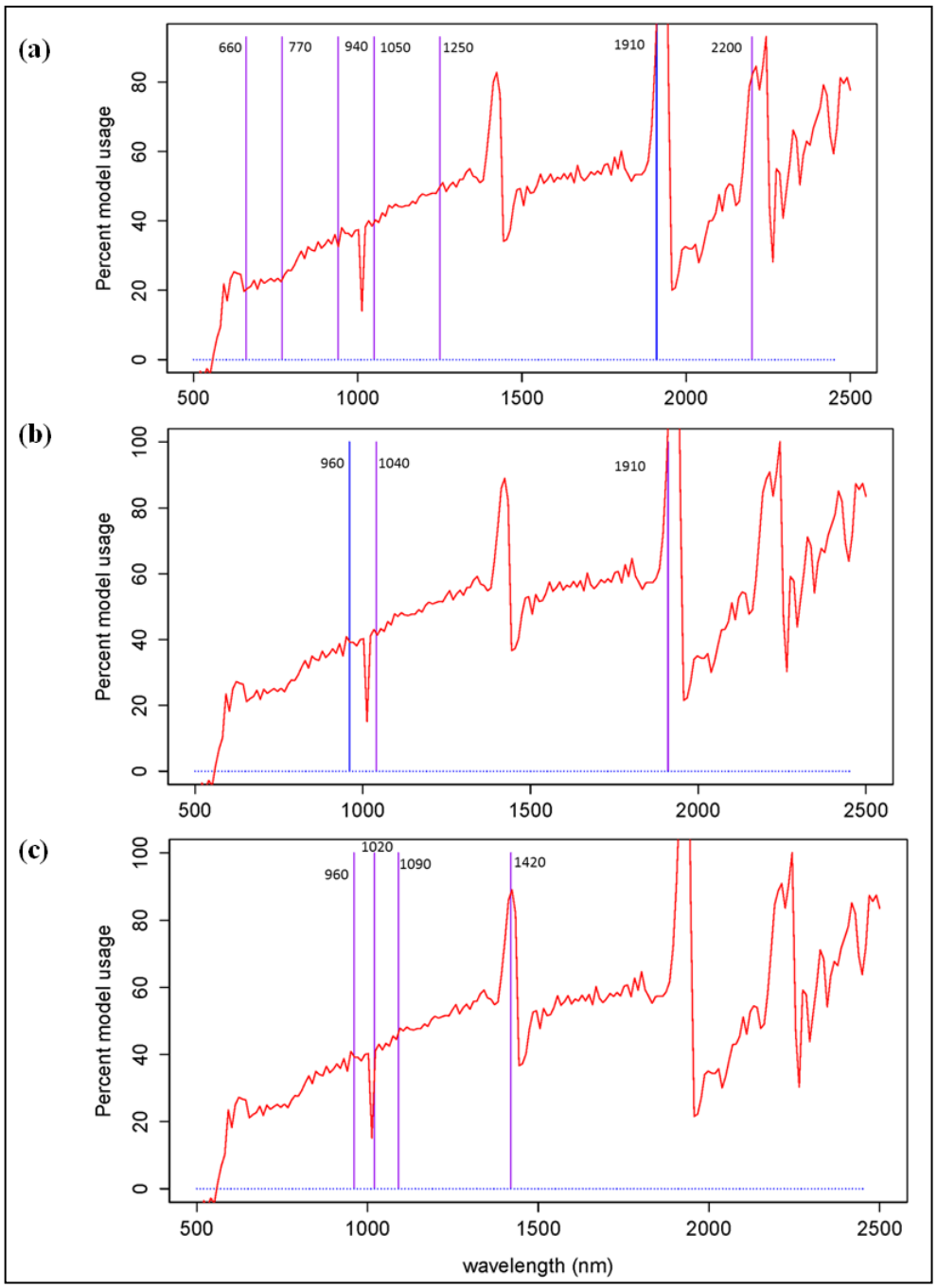


Figure 3.7. Important variables used in the Cubist model by bulk soil Vis NIR spectra to predict carbon in macroaggregates (a), microaggregates (b) and organo-mineral associations (c).

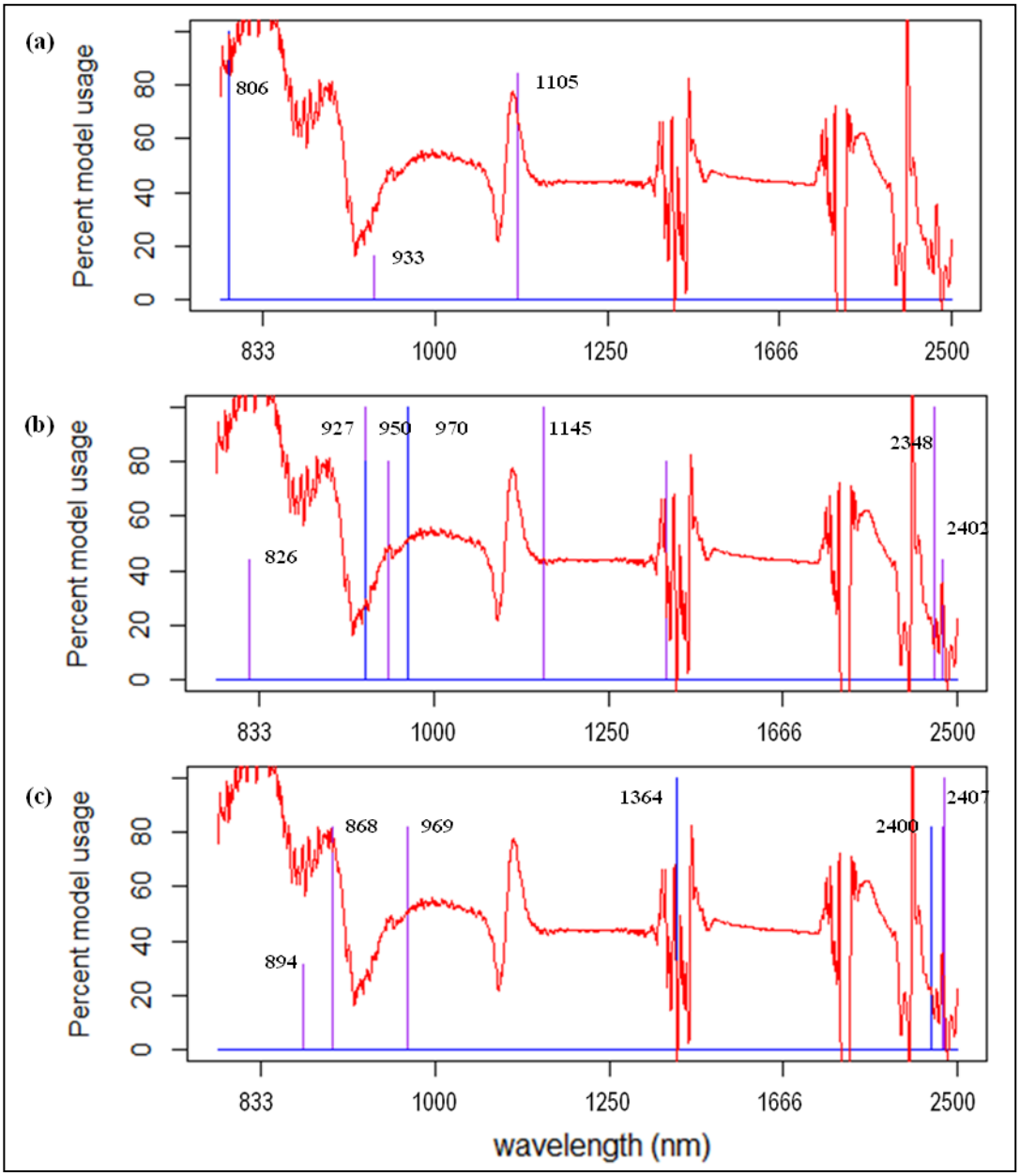


Fig. 3.8. Important variables used in the Cubist model by bulk soil NIR spectra to predict carbon in macroaggregates **(a)**, microaggregates **(b)** and organo-mineral associations **(c)**.

Table 3.4. Important absorptions bands derived by bulk MIR spectra and relevant functional groups.

Wavelength (nm)	**Possible functional groups**
Macroaggregates	
12626	Aromatic C-H (12903 nm)[a]
10787	Fe/Al oxides (10869 nm)[b] Polysaccharides (10526-8547 nm)[c] Aromatics and light fraction OM (10928 nm)[d]
7097	Aliphatic-CH (7092 nm)[h]
3676	Aliphatic C-H [f]
2699	Clay minerals (2674 nm)[f]
Microaggregates	
14306	Iron oxides (14285-16666 nm)[f]
12626	Aromatic CH (12903 nm)[a]
9416	Polysaccharides (10526-8547 nm)[c]
5232	Quartz overtones (5586-5000 nm)[f]
4882	Quartz overtones (5586-5000 nm)[f]
3756	Inorganic carbon (calcite and dolomite) (3972 nm)[f]
2631	Clay minerals (2710 nm)[f]
Organo-mineral associations	
9960	Silicates and clay minerals (10000-9090 nm)[f]
9587	Silicates and clay minerals (10000-9090 nm)[f]
6435	Amide II bands(6426 nm)[g]
5232	Quartz overtones (5586-5000 nm)[f]
5181	Quartz overtones (5586-5000 nm)[f]
4887	Carbohydrates (4728 nm)[e]
3824	Aliphatic C-H[f]

[a]Baes and Bloom (1989)
[b]Haberhaueret al. (1998)
[c]Stevenson (1994)
[d]Janik et al. (1998)
[e]Rumpel et al. (2001)
[f]Calderon et al. (2011)
[g]Janik et al. (2007)
[h]Solomon et al. (2005)

Table 3.5. Important absorptions bands derived by bulk NIR spectra and relevant functional groups.

Wavelength (nm)	Possible functional groups
Macroaggregates	
806	Aromatics (825 nm) Amine (751 nm)
933	Hydroxyl groups (930 nm)
1105	Organic aromatic (1100 nm)
Microaggregates	
826	Aromatics (830 nm)
927	Geothite (920 nm)
950	Water (940 nm) Hydroxyl groups (930 nm)
1145	Aromatics (1100)
1364	Kaolinite (1395 nm)
2348	Carbohydrates (2381 nm)
2402	Carbohydrates (2381 nm)
Organo-mineral associations	
868	Alkyl C-H (853 nm and 877 nm)
894	Alkyl C-H (853 nm and 877 nm)
969	Geothite (940 nm)
1382	Kaolinite (1395 nm)
2327	Carbohydrates (2381 nm)
2400	Illite (2450 nm)
2407	Illite (2450 nm)

Viscarra-Rossel and Behrens (2010).

Table 3.6. Important absorptions bands derived by bulk Vis-NIR spectra and relevant functional groups.

Wavelength (nm)	**Possible functional groups**
Macroaggregates	
660	Iron oxides (650 nm)
770	Amine (751 nm)
940	Water (940 nm)
1050	Organic aromatic (1100 nm)
1250	Aliphatic compounds
1910	Water (1915 nm) Carboxylic acids (1930 nm)
2200	Aliphatic compounds (2227 nm)
Microaggregates	
960	Water (940 nm)
1040	Organic aromatics (1100 nm)
1910	Water (1915 nm)
Organo-mineral associations	
960	Water (940 nm)
1020	Organic aromatic (1100 nm)
1090	Organic aromatic (1100 nm)
1420	Clay minerals – Kaolin (1415 nm)

Viscarra-Rossel and Behrens (2010).

3.3.4.2. Use of absorption differences of mean spectra of aggregate fractions

Figure 3.12 (a), (b) and **Figure 3.13** shows the absorption differences derived from mean aggregate fraction spectra subtracted from the mean bulk soil Vis-NIR, NIR and MIR spectra. The MIR peaks are much greater and show more peaks than NIR, as the MIR spectra always show relatively more features than the NIR spectra (Reevs, 2003). The highest absorbance differences were observed in macroaggregate fractions at wavelengths 8956 nm, 8503 nm, and 8079 nm and these absorption bands are related to polysaccharides (Calderón et al., 2011). In fact, Sarkoht et al. (2007) reported that the peak at 8503 nm found in soil aggregates could be due to the polysaccharides in aggregate fractions (Calderón et al., 2011). There are also two polysaccharides peaks observed at 9749 nm and 4000-4700 nm. The high absorption peak found at 10672 nm is possibly due to the presence of carbohydrates. The peak at 6045 nm represents the functional groups of lignin, proteins and humic acids (Calderón et al., 2011). Absorption differences of macroaggregates spectra derived from Vis-NIR and NIR showed fewer peaks and these were also related with aliphatic and hydroxyl groups (Viscarra- Rossel and Behrens, 2010). Hydroxyl compounds are mainly alcoholic, carbohydrates and phenolic substances. These results are greatly supported by the wavelength used by the Cubist model to predict macroaggregate-C from bulk soil spectra (**Table 3.4, 3.5 and 3.6**). According to the both spectral analyses, macroaggregates were predominantly enriched by polysaccharides, carbohydrates and aliphatic compounds. The reason being macroaggregates are associated with fungal hyphae, bacterial cells, algae and other transformation products such as aliphatic compounds (Jastrow and Miller, 1998; Tisdall and Oades, 1982). Thus, when this fraction is predicted from the bulk soil spectra, these functional groups are used as a predictor. Also there were lignin and phenolic bands observed in both MIR and Vis-NIR spectra. Monreal et al. (1995) found that the proportion of macroaggregates was highly correlated with lignin dimers. This suggests that thepresence of lignin in macroaggregates could be due to the effect of plant roots on the temporal stability of macroaggregates as proposed by Oades and Waters (1991).

The microaggregate absorptions differences are located between macroaggregates and organo-mineral associations (**Figure 3.12 (a), (b)** and **Figure 3.13**). The spectral peaks of microaggregates are similar to that of macroaggregates; however, the absorption difference for microaggregates was much lower than macroaggregates. Macroaggregates are formed by microaggregates and bound together with transient and temporary binding agents (Tisdall and Oades, 1982). Thus, macroaggregate fraction contains both microaggregate and macroaggregate associated C. According to both the Cubist predicted wavelengths and absorption differences of mean macroaggregate spectra, the microaggregated-C is predicted mainly from the absorption regions related to aromatic compounds and polysaccharides. Thus, microaggregates are believed to be stabilized mainly by persistent agents such as aromatic humic materials associated with polyvalent metal cations such as amorphous iron and aluminum oxides and polysaccharides (Jastrow and Miller, 1998). There are small peaks observed at 14368 nm and 7057 nm is due to the presence of iron oxides and carboxyl-C as followed (Rumpel et al., 2001). During the SOM stabilization process, microaggregates has shown coated with carboxyl-C of exterior of the aggregates and interior region is consistent with more aromatic and aliphatic C (Lal et al., 2009)

The mean absorption difference of organo-mineral associations showed both positive and negative peaks with respect to bulk MIR and Vis-NIR spectra. This fraction mainly consists of clay and silt particles associated with micro-organisms, fine roots and plant materials (Jasrow and Miller, 1998). There were negative peaks observed at 8130 nm and 2734 nm due to the presence of high amounts of clay minerals in this fraction compared to the bulk soil (**Figure 3.13**). The peak at 3091 nm indicates the presence of iron oxides and aromatic compounds (Calderón et al., 2011). Absorption of quartz overtones were observed at 5543 nm, 5268 nm and 4980 nm (Calderón et al., 2011). The observed sharp peak at 7134 nm is due to the presence of aliphatic compounds of the organo-mineral associations. Mean absorption differences of organo-mineral associations was significantly markly high within the region 500 nm -1400 nm of the Vis NIR spectra. This region mainly consists of aromatic compounds and iron oxides

bands (Viscarra Rossel and Behrens, 2010). The Cubist predicted wavelengths of both MIR and Vis-NIR showed similar functional groups relevant to this fraction. Previous studies revealed that SOM associated with organo-mineral associations was enriched with various organic functional groups. Amide forms, aliphatic-C and oxidized-C are dominant in phyllosilicate, quartz, feldspar and iron oxides groups. Thus, our results show that amide-C (6435 nm) have significantly influenced predictions of carbon in this fraction (**Table 3.5**). Also Sarkhot et al. (2007) observed amides peak for > 53 μm wet- and dry-sieved derived aggregate fractions by MIR spectra. The wavelength corresponding to weathered mineral soil was shown by the pronounced iron oxide and clay mineral features near 900 nm (Visacarra-Rossel and Webster, 2012). Wavelengths around 9960 nm, 9587 nm of MIR and 1420 nm of NIR correspond to silicates and clay minerals since this fraction is enriched with coarse silt, coarse clay and fine clay (Christensen, 1996).

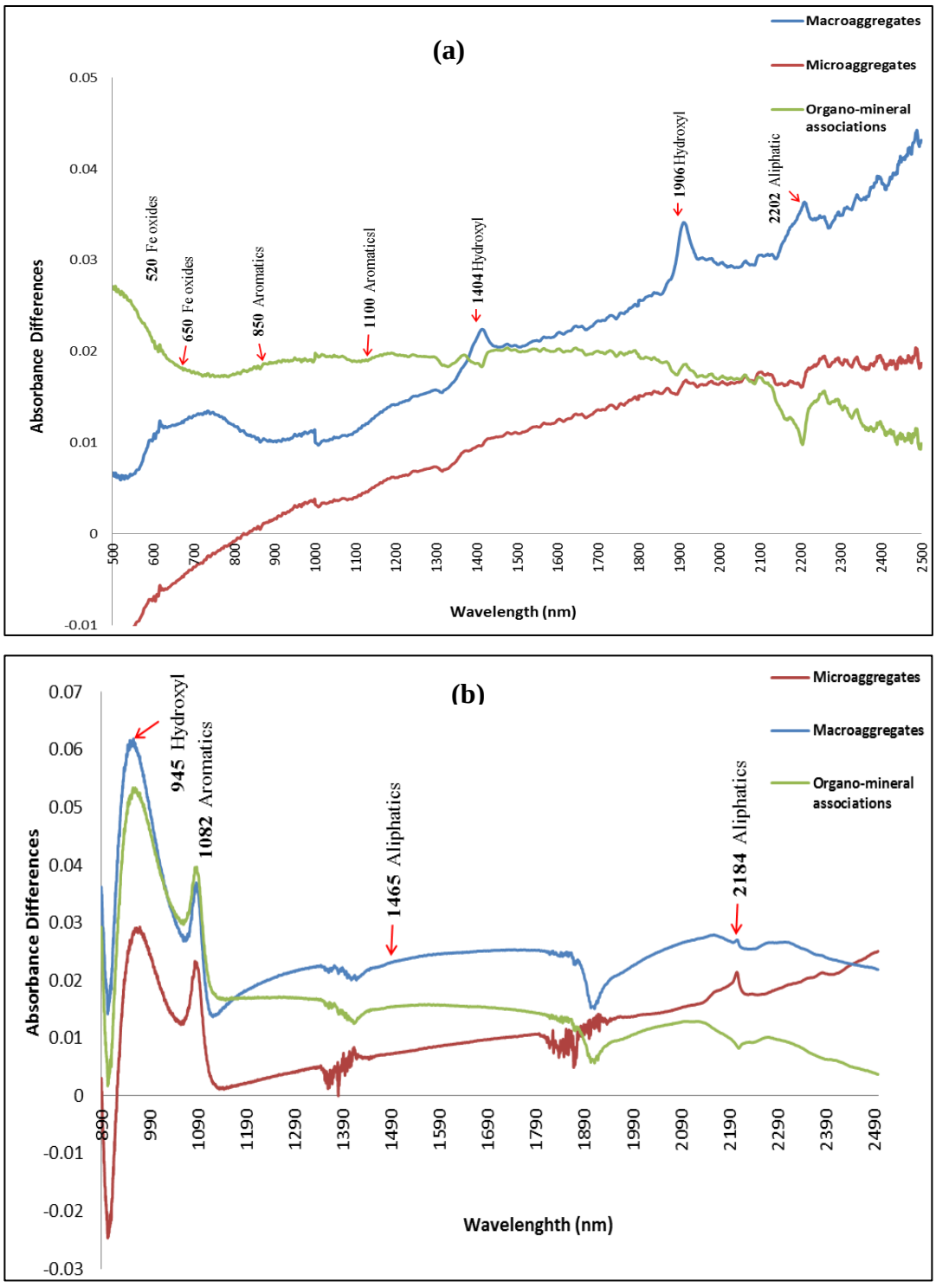


Figure 3.9. The absorption differences of mean Vis-NIR (a) and NIR

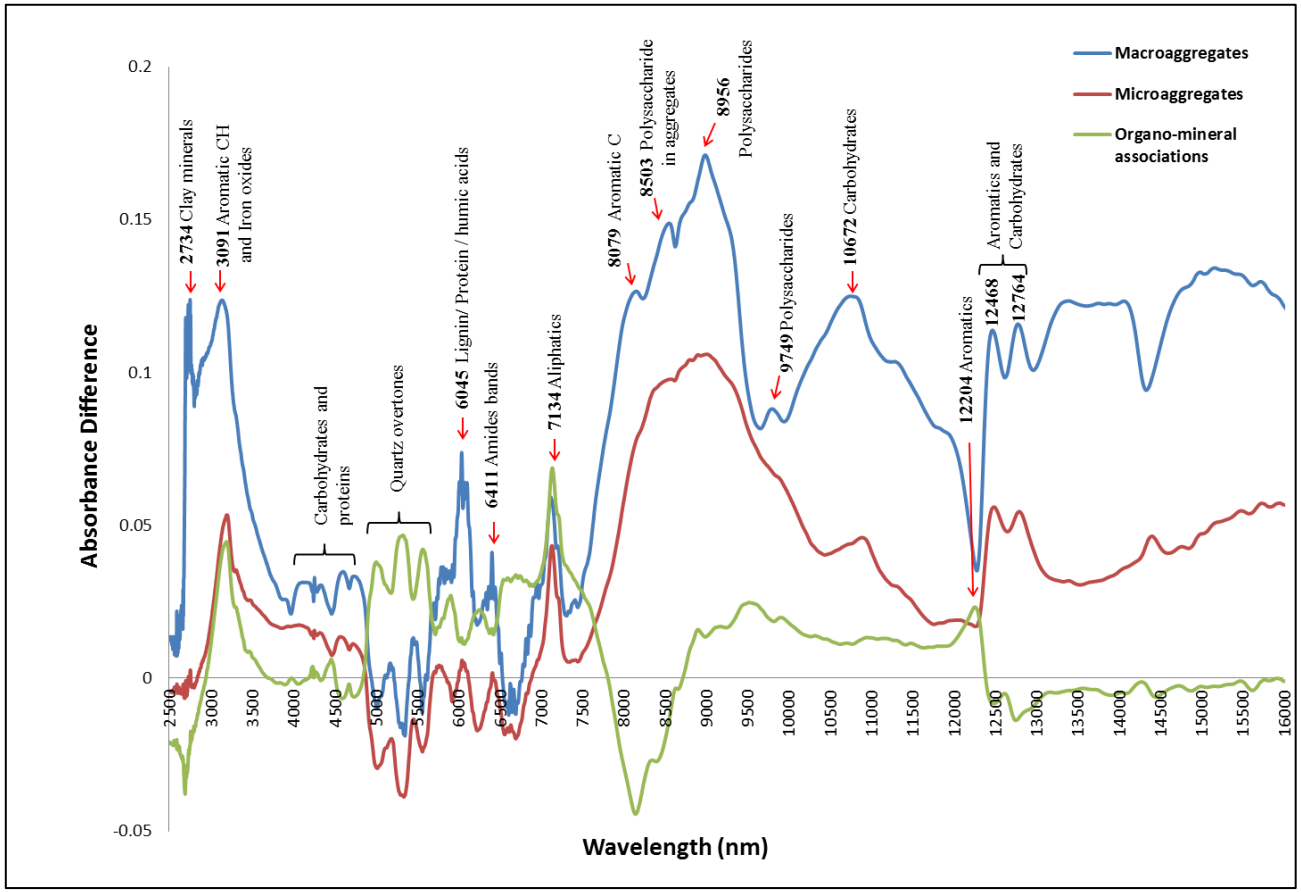


Figure 3.10. The absorption differences of mean MIR spectra of macroaggregates, microaggroaggregates and organo-mineral associations.

3.4. Conclusions

MIR has a greater potential to quantify the aggregate-C than NIR spectroscopy and this can be explained by MIR having more fundamental absorption features. NIR produced slightly better results than Vis-NIR predictions which were particularly poor. Vis-NIR has been reported to have a greater potential to predict most soil properties, yet this was not confirmed in the results presented here when considering aggregated-C. This may be due to the more light diffusion that can affect NIR caused by the physical structure (size of aggregates, porosity) and/or presence of water changing the refractive index of the samples being analysed (Williams and Norris, 1987). The important wavelength used by the Cubisit model and the use of mean absorption of the aggregate spectra well explained the specific functional groups contained in the aggregate fractions. Thus, the proposed method to quantify soil aggregate C fractions from bulk soil MIR spectra can be very helpful in analyzing large number of samples without recourse to time-consuming traditional fractionation methods.

3.5. References

Amezketa, E., 1999. Soil aggregate stability: a review. Journal of Sustain Agriculture. 14, 83-151.

Ashman, M.R., Hallett, P.D., Brookes, P.C., 2003. Are the links between soil aggregate size class, soil organic matter and respiration rate artefacts of the fractionation procedure?. Soil Biology and Biochemistry. 35, 435-444.

Baes, A.U., Bloom, P.R., 1989. Diffuse reflectance and transmission Fourier transformation infrared (DRIFT) spectroscopy of humicand fulvic acids. Soil Science Society of America Journal. 53, 695-700.

Bellon-Maurel, V., McBratney, A., 2011.Near-infrared (NIR) and mid-infrared (MIR) spectroscopic techniques for assessing the amount of carbon stock in soils – Critical review and research perspectives. Soil Biology and Biochemistry. 43, 1398-1410.

Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, P., Roger, J.M., McBratney, A.B., 2010. Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. Trends in Analytical Chemistry. 29, 1073-1081.

Bronick, C.J., Lal R., 2005. Soil structure and management: a review. Geoderma**.** 124, 3-22.

Calderón, F.C., Reeves III, F.J., Collins, H.P., Paul, E.A., 2011. Chemical differences in soil organic matter fractions determined by Diffuse-Reflectance Mid-Infrared spectroscopy. Soil Science Society of American Journal. 75, 568-579.

Cambardella, C.A., Elliott, E.T., 1994. Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Science Society of America Journal. 58, 123-130.

Carter, M.R., 1996. Analysis of soil organic matter storage in agro ecosystems. In: Stewart, B.A. (Eds.), Structure of Organic Matter Storage in Agricultural Soils. CRC Press Inc, Boca Raton, FL, pp3-11.

Chang, C.W., Laird, D.A., Mausbach, M.J., Hurburgh C.R., J., 2001 Near-infrared reflectance spectroscopy-Principal components regression analyses of soil properties. Soil Science Society of America Journal. 65, 480-490.

Christensen B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science. 52, 345-353.

Davies, A., Fearn, T., 2006. Back to basics: calibration statistics. Spectroscopy Europe 18, 31-32.

Janik, L.J., Forrester, S.T., Rawson, A., 2009. The prediction of soil chemical and physical properties from mid-infrared spectroscopy and combined partial least-squares regression and neural networks (PLS-NN) analysis. Chemometrics and Intelligent Laboratory Systems. 97, 179-188.

Janik, L.J., Merry, R.H., Skjemstad, J.O., 1998. Can mid-infrared diffuse reflectance analysis replace soil extractions?. Australian Journal of Experimental Agriculture. 38, 681-696.

Jastrow, J.D., Boutton, T.W., Miller, R.M., 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Science Society of America Journal. 60, 801-807.

Jastrow, J.D., Miller, R.M., 1998. Soil aggregate stabilization and carbon sequestration. P. 207-233. In Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A., (Eds.), Soil Processes and the Carbon Cycle. CRC Press, Boca Raton, FL. pp207-223.

Kuhn, M., Weston, S., Keefer, C., Coulter, N., 2013. Cubist package. Version 0.0.13

Kaewmano, C., Kheoruenromne, I., Suddhiprakarn, A., Gilkes, R.J., 2009. Aggregate stability of salt-affected kaolinitic soils on the North-east Plateau, Thailand. Soil Research. 47, 697-706.

Madari, B.E., Reeves III, J.B., Coelho, M.R., Machado, P.L.O.A., De-Polli, H., Coelho, R.J., Benites, V.M., Souza, L.F., McCarty, G.W., 2005. Mid- and near-infrared spectroscopic determination of total and organic carbon in a diverse set of soils from the Brazilian national soil collection. Spectroscopic Letters. 38, 721-740.

McCarty, G.W., Reeves, J.B., Reeves, V.B., Follett, R.F., Kimble, J.M., 2002. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. Soil Science Society of America Journal. 66, 640-646.

McBratney, A.B., Minasny, B., Viscarra-Rossel, R., 2006. Spectral soil analysis and inference systems: A powerful combination for solving the soil data crisis. Geoderma. 136, 272-278.

Minasny, B., McBratney, A.B., 2006. A conditioned Latin hypercube method for sampling in the presence of ancillary information. Computers and Geosciences. 32, 1378-1388.

Minasny, B., McBratney, A.B., 2008. Regression rules as a tool for predicting soil properties from infrared reflectance spectroscopy. Chemometrics and Intelligent Laboratory Systems. 94, 72-79.

Minasny, B., McBratneya, A.B., Stockmanna, U., Hongb, Y.S., 2013. Cubist, a regression rule approach for usein calibration of NIR spectra. NIR 2013 Proceedings, 2-7 June, La Grande-Motte, France.

Minasny, B., McBratney, A.B., 2013. Why you don't need to use RPD. Pedometron. 33, 14-15.

Monreal, C.M., Schnitzer, M., Schulten, H.R., Campbell, C.A., Anderson, D.A., 1995. Soil organic structures in macro and microaggregates of a cultivated Brown Chernozem. Soil Biology and Biochemistry. 27, 845-853.

Puget, P., Chenu, C., Balesdent, J., 1995. Total and young organic matter distributions in aggregates of silty cultivated soils. European Journal of Soil Science. 46, 449-459.

Reeves III, J.B., 2003. Mid-infrared diffuse reflectance spectroscopy: is sample dilution with KBr necessary, and if so, when?. American Laboratory. 35, 24-28.

Rumpel, C., Janik, L.J., Skjemstad, J.O., Kögel-Knabner, I., 2001. Quantification of carbon derived from lignite in soils using mid-infrared spectroscopy and partial least squares. Organic Geochemistry. 32, 831-839.

Sarkhot, D.V., Comerford, N.B., Jokela, E.J., Reeves III, J.B., Harris, H., 2007. Aggregation and gggregate carbon in a forested south eastern coastal plain Spodosol. Soil Science Society of America Journal.71, 1779-1787.

Sanderman, J., Baldock, J., Hawke, B., Macdonald, L., Massis-Puccini, A., Szarvas, S., 2011. National soil carbon research programme: Field and Laboratory Methodologies, CSIRO.

Singh, K., Minasny, B., McBratney, A.B., Short, M.G., Namazi, F.M., 2012. Sampling for field measurement of soil carbon using Vis-NIR spectroscopy. 5th Global workshop on Digital Soil Mapping 2012 conference. Sydney, 10-3 April 2012.

Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000. Soil structure and organic matter: I. distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal. 64, 681-689.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil. 241, 155-176.

Stevenson, F.J., 1994. Humus Chemistry. Genesis, Composition, Reactions, 2nd edition. Wiley, New York.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils.Journal of Soil Science. 33, 141-163.

Tidall, J.M., 1996. Formation of soil aggregates and accumulation of soil organic matter. 57-96. In: Carter, M.R., Stewart, B.A., (Eds.), Structure and Organic Matter Storage in Agricultural Soils. CRS Press, Inc., Boca Raton, FL. pp57-96.

Viscarra-Rossel, R.A., Walvoort, D.J.J., McBratney, A.B., Janik, L.J., Skjemstad, J.O., 2006. Visible, near infrared, mid-infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. Geoderma. 131, 59-75.

Viscarra-Rossel, R.A., Behrens, T., 2010. Using data mining to model and interpret soil diffuse reflectance spectra. Geoderma. 158, 46-54.

Williams, P.C., 1987. Variables affecting near-infrared reflectance spectroscopic analysis. In: Williams, P.C., Norris, K.H., (Eds.), Near-Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemists, St Paul, MN. pp143-167.

Williams, P., 2003. Near-infrared technology-getting the best out of light. PDK Projects, Nanaimo, Canada.

Wheeler, I., Minasny, B., McBratney, A.B., Bui, E., Wilford, J., Lymburner, L., 2013. Three-dimensional spatial prediction of soil organic carbon for three contiguous bioregions in New South Wales, Australia. European Journal of Soil Science (in press).

Chapter 4: Investigating the potential to predict carbon pools in aggregate fractions using mid infra-red spectroscopy.

4.1. Introduction

SOM is a heterogeneous compound consisting of various functional pools that are stabilized by specific mechanisms defined by different turnover rates. The soil organic carbon (SOC) that comprises SOM is divided into carbon pools that reflect the different turnover rates, which in turn effect soil quality and function (Baldock and Skjemstad, 1999; Jenkinson et al., 1990; Parton et al., 1987). For example, the labile pool provides a source of energy and nutrients to the decomposer community and the stable pool acts as a reservoir to maintain long-term soil productivity. To ensure the optimum productivity of soils, it is not only essential that adequate levels of SOC must be maintained, but also that adequate levels of the correct compounds of SOC are present (Baldock and Skjemstad, 2000).

Quantification of soil carbon pools is important for understanding SOC dynamics by means of soil carbon turnover models (Krull et al., 2003). The carbon turnover models developed are based on several conceptual pools which differ from each other by decomposition rates. There are a few SOC models that have incorporated soil physical measures, beyond a sample measure of bulk density, to quantify the formation of soil aggregates in correlation with SOC dynamics (Gryze et al., 2006; Malamoud et al., 2008: Segoli et al., 2013). According to the aggregate hierarchy model (Tisdall and Oades, 1982), primary particles are cemented together to form microaggregates and these are bound together into macroaggregates. There are a range of binding materials involved in aggregate formation. Microaggregates are stabilized mainly by persistent agents (aromatic humic materials associated with polyvalent cations) and perhaps some transient materials (polysaccharides-polymers derived from plants and microorganisms), and macroaggregates are bound together by transient and temporary (roots, fungal

hyphae, bacterial cells and algae) agents (Jastrow and Miller, 1998). This presence of different binding materials of different chemical compositions explains the difference in turnover rates. Over the past two decades, numerous studies have been conducted to find out how these different functional pools form and stabilize in soil aggregates (Camberdella and Elliott, 1992; Jastrow et al., 1996; Six et al., 2002). For example, the active pool is significantly important the formation of macroaggregates, while clay+silt associated SOC is important for the microaggregate stabilization. Consequently, these relationships that have facilitated the development of aggregate turnover models to explain the both aggregation and SOC turnover together (Gryze et al., 2006; Malamoud et al., 2008: Segoli et al., 2013).

The major limitation of all these soil carbon and aggregate turnover models is that SOC pools defined within these models are conceptual in nature (Krull, et al., 2003). However, recent studies have made attempts to use measurable fractions to initialize SOC pools of SOC models (Skjemstad et al., 2004; Segoli et al., 2013; Zimmermann et al., 2007a). Numerous methods have been used to fractionate soil samples into different C pools by physical and chemical fractionation procedures. Most of the laboratory-based SOC fractionation schemes remain time-consuming and require specialized analytical equipment, and they sometimes do not correspond to specific stabilization mechanisms and hence do not describe functional SOC pools (Baldock et al., 2013; von Lützow et al., 2007). As an example, density separation of the light fraction particulate organic matter (LF-POM) and heavy fraction POM (Sohi et al., 2001) requires 2-3 days depending on the physical and chemical nature of the soil sample. If the sample contains more LF-POM, it will need additional days for the separation and removal of sodium polythungstate (SPT) from the soil fractions. Not only the time but also SPT is very expensive and an environmentally hazardous chemical (Six et al., 1999). Thus, the use of traditional fractionation procedures is not feasible to analyze large numbers of samples in routine soil analysis. Therefore, spectroscopic techniques are important quantification methods to overcome the limitations described above as they were shown more rapid and cost effective. MIR measurements were shown reliable predictions of

wide range of soil chemical and physical properties (Janik et al. 1998; McCarty et al. 2002; Viscarra Rossel et al. 2006). However, relatively few studies showed appropriate predictions for quantification of SOC pools by using NIR and MIR techniques (Baldock et al., 2013; Reeves et al. 2006; Reeves et al., 2008; Janik et al. 2007; Zimmermann et al. 2007b).

There is a no previous study has reported the use of spectroscopic techniques to quantify soil carbon pools which represent the functional groups in aggregate fractions. It is meaningful to develop combined carbon and aggregate turnover models with measurable fractions and would be of great benefit to find reliable cost and time efficient methods to avoid conventional fractionation methods including wet sieving, physical and chemical fractionation procedures. As we concluded in **Chapter 3**, MIR was shown successful to quantify aggregate carbon from bulk soil spectra. Thus, the objective of this study is to investigate the potential use of MIR bulk soil spectra to predict individual SOC fractions of aggregate fractions.

4.2. Materials and methods

Soil sampling, aggregate fractionation and initial soil analysis were done according to the methods described in **sections 3.3.1, 3.3.2** and **3.3.3** respectively.

4.2.1. Soil organic carbon fractions separation

Both bulk soil and aggregate fractions were separated into soil carbon pools using combined physical and chemical fractionation method described by Zimmermann et al., (2007a) (**Figure 4.1**)

4.2.1.1. Physical fractionation procedure

Thirty grams of bulk soils and aggregate frcations were dispersed with 150 ml of water using an ultrasonic probe (Hielscher Ultrasound Technology, Teltow, Germany) with an output energy of 22 Jml^{-1} to maximize the dispersion and minimize the disruption of mineral fractions. Amelung and Zech (1999) reported that anultrasonic dispersion energy of > 3 Jml^{-1} is required to disrupt macroaggregates, and $\geq$ 5 Jml^{-1} is required to disrupt microaggregates. The energy levels > 25 Jml^{-1} disrupted the coarse-sand size fraction. The dispersed suspension was wet sieved on a 63 µm sieve until the rinsing water turned clear. The suspension < 63 µm was filtered by using millipore apertures with 0.45 µm nylon mesh. The filtrate (dissolved organic matter) was stored in a freezer at a temperature of -4 °C. The solid fractions were oven dried at 40 ^{0}C and weighed.

4.2.4.2. Chemical fractionation procedure

The fraction > 63 µm was used for the density fractionation (Sohi et al., 2001). This fraction was mixed with sodium polytungstate at a density of 1.8 g cm^{-3} and centrifuged at 1000g for 15 minutes. The floating fraction (light fraction) was separated. The light fraction (LF-POM) was washed six times with deionised water using a milipore apparatus system. The remaining fraction > 63 µm (S+A) consisted of sand and stable aggregates and this was washed with deionised water using 63 µm size nylon bags. One gram of the fraction > 45 µm (S+C) was oxidized with 50 ml of 6% sodium hypochloride adjusted to pH 8 with concentrated HCl to separate the RSOC fraction. The mixture was shaken for 18 hours at 25 ^{0}C using electrical shaker. The oxidized residue was centrifuged at 1000 g for 15 minutes and the upper liquid portion removed. The solid fraction was washed with deionized water and centrifuged again. This oxidation step was repeated twice. All solid fractions were oven dried at 40 ^{0}C.

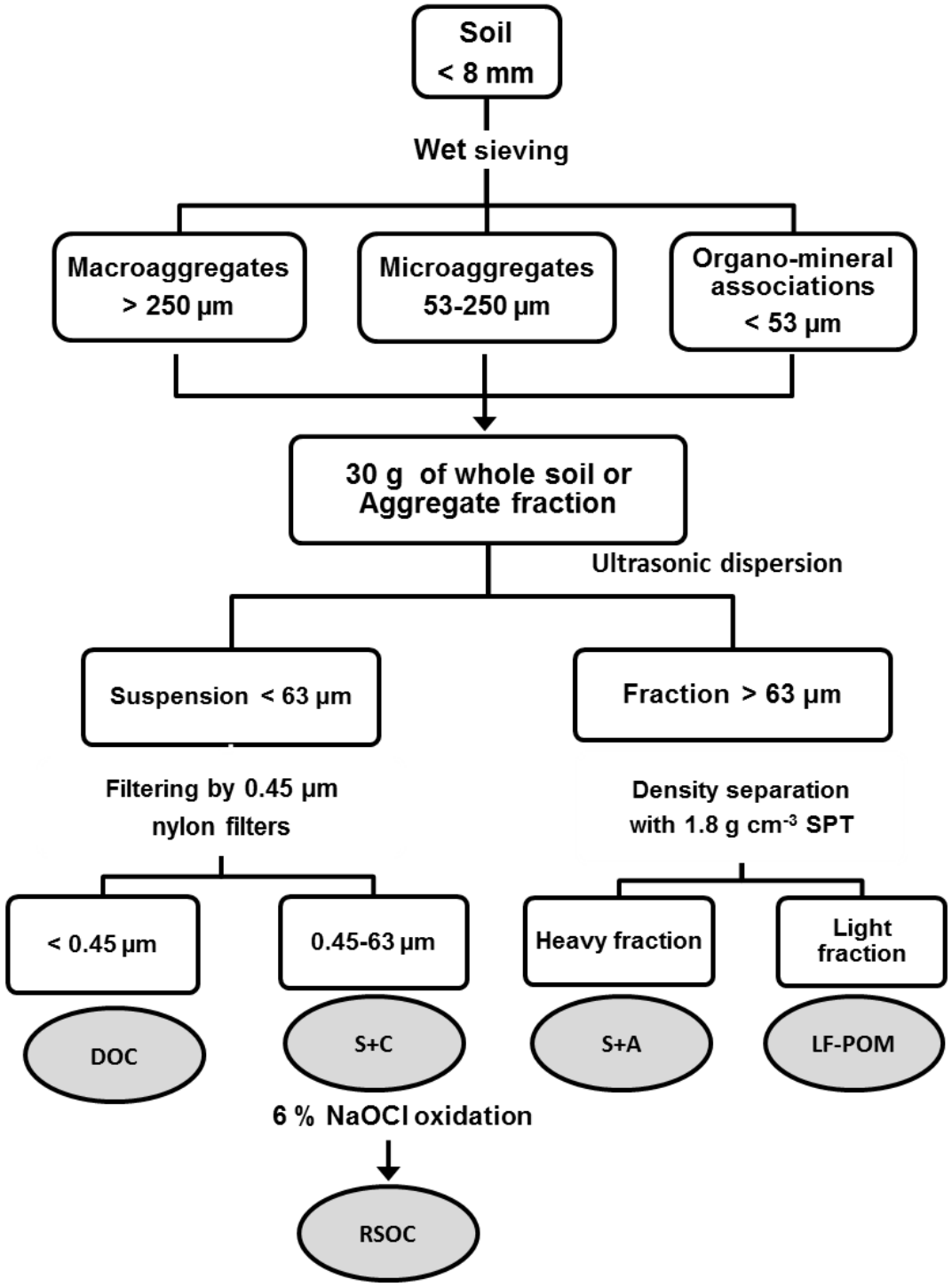


Figure 4.1. Combined fractionation scheme used for separation of aggregate fractions and SOC fractions.

4.2.2. Measurement of TOC

The TOC of the solid fractions were measured by dry combustion using a Vario Max CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and the liquid fractions (DOC) were analyzed using a Shimadzu TOC-VCSH analyser.

4.2.3. Calculation of mass and carbon recoveries

Mass recovery and carbon pools recoveries of bulk soil samples were calculated by the equations mentioned below.

$$\text{Mass Reccoveries \%} = \frac{\text{Dry mass } ((\text{S} + \text{C}) + (\text{LF} - \text{POM}) + (\text{S} + \text{A}) + (\text{RSOC}) + (\text{DOC}))}{\text{Dry mass of Total soil}} \times 100 \quad [4.1]$$

$$\text{Carbon recovery \%} = \frac{\text{Carbon \%} \ \ ((\text{S} + \text{C}) + (\text{S} + \text{A}) + (\text{LF} - \text{POM}) + (\text{DOC}))}{\text{Carbon \% of Bulk soil}} \times 100 \quad [4.2]$$

4.2.4. Spectroscopic measurement as analysis

Bulk soil MIR spectra recording, spectra analysis and cubist model evaluation were done according to the methods described in sections **3.2.4** and **3.2.6** and **3.2.8** respectively.

4.3. Results and Discussion

4.3.1. Mass and carbon recoveries

The basic soil properties were described in **Chapter 3.** 98.91% mass mean recovery and 98.58% carbon recovery were achieved. The scatter plot for bulk soil TOC and sum of C % of S+C, S+A, LF-POM, RSOC and DOC is illustrated in **Figure 4.2.** The accuracy of the fractionation procedure can be determined by the mass recovery % and carbon recovery % of the samples. Mass recovery > 98% and carbon recoveries of 85-115% support the use of the fractionation procedure (Sanderman et al., 2011).

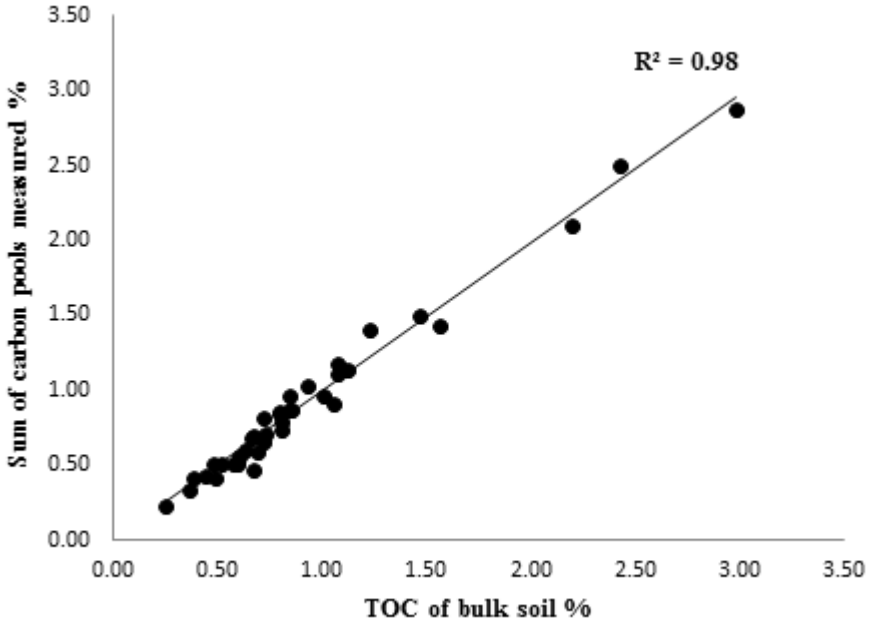


Figure 4.2. Relationship between TOC of bulk soil and sum of S+C, S+A, LF-POM, RSOC and DOC.

4.3.2. Carbon pool distribution of bulk soil

Carbon distribution into SOC fractions is summarized in **Figure 4.3**. The S+C fraction was the largest carbon pool with 68% of the TOC. The carbon contents of S+A, LF-POM RSOC and DOC were 10.15%, 10.15%, 9.13% and 1.52% respectively. These results of measured S+C, S+A, LF-POM, RSOC and DOC fractions are in the range of the studies of Poeplau and Don (2013) and Wiesmeier et al. (2014) who fractionated soils from different land use systems according to the method of Zimmermann et al. (2007a). The S+C fraction can protect more carbon by adsorbing organic molecules to the clay mineral structure (Christensen, 2001). Generally, about 50-75% of total OC was associated with clay-sized particles (< 2 µm) and 20-40% with silt sized particles (2-63 µm) in temperate arable soils (von Lützow et al., 2007). LF-POM and DOC fractions are considered as the most labile pools and these fractions are very sensitive to land use systems and management practices (Poeplau and Don, 2013).

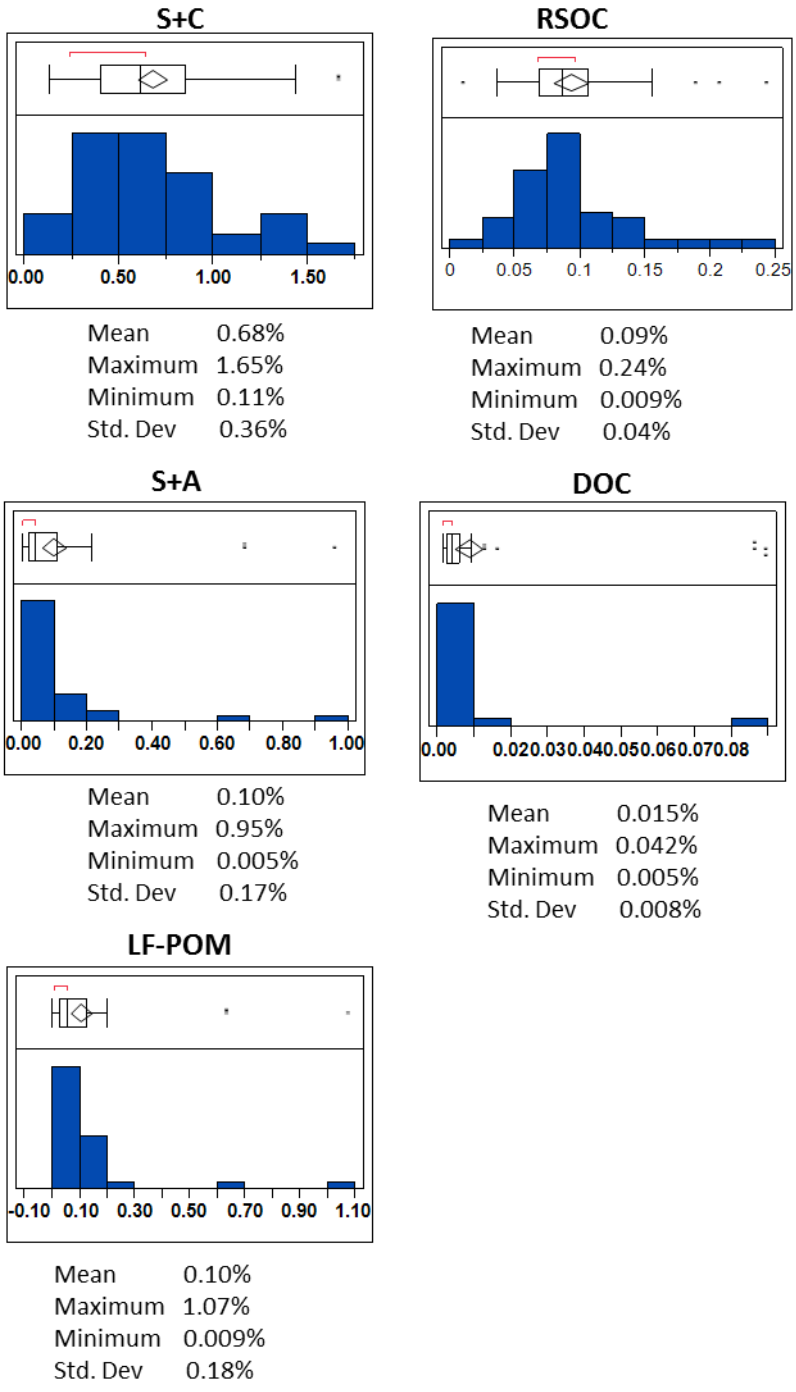


Figure 4.3. C distribution and summary statistics of measure soil carbon fractions of bulk soils.

4.3.3. Prediction of the carbon pools of bulk soils.

Figure 4.4. shows the leave-one-out cross validation results using MIR spectra of bulk soil samples. The Cubist predictions for S+C, S+A and LF-POM fractions were accurately predicted by MIR spectra. R^2 was 0.79 for S+C fractions with a RPIQ of 1.35. However the RMSE value (0.19) for S+C was the highest among all fractions. The Cubist prediction for LF-POM fraction was excellent with R^2 = 0.91, RMSE = 0.08 and RPIQ = 0.56. The correlation coefficient of measured and predicted carbon in S+A fraction was 0.90 with RMSE = 0.08 and RPIQ = 0.34. Reeves et al. (2006) and Zimmermann et al. (2007b) have shown accurate predictions for S+C and LF-POM pools from much diversified data sets using the PLS approach. The predictions for RSOC gave R^2 = 0.72 but with low RMSE % and a high RPIQ value. However, unsatisfactory predictions were observed for the DOC fraction. Zimmermann et al. (2007b) also reported low prediction results for the DOC fraction. They reasoned the poor prediction to be due to the fractionation procedure. According to the fractionation procedure, it is difficult to maintain a constant amount of water. Furthermore, DOC accounts for a very small amount of carbon compared with other fractions. However, this study tried to minimize the error of the fractionation procedure by keeping a constant amount of water at the end of the fractionation. Further, as the carbon distribution showed a wide variability among the fractions, we used the Cubist models as the calibration method instead of PLS. Cubist was shown to have a better prediction abilitiesthan PLS when using small concentrations as well as high concentrations of carbon (Minasny et al., 2013).

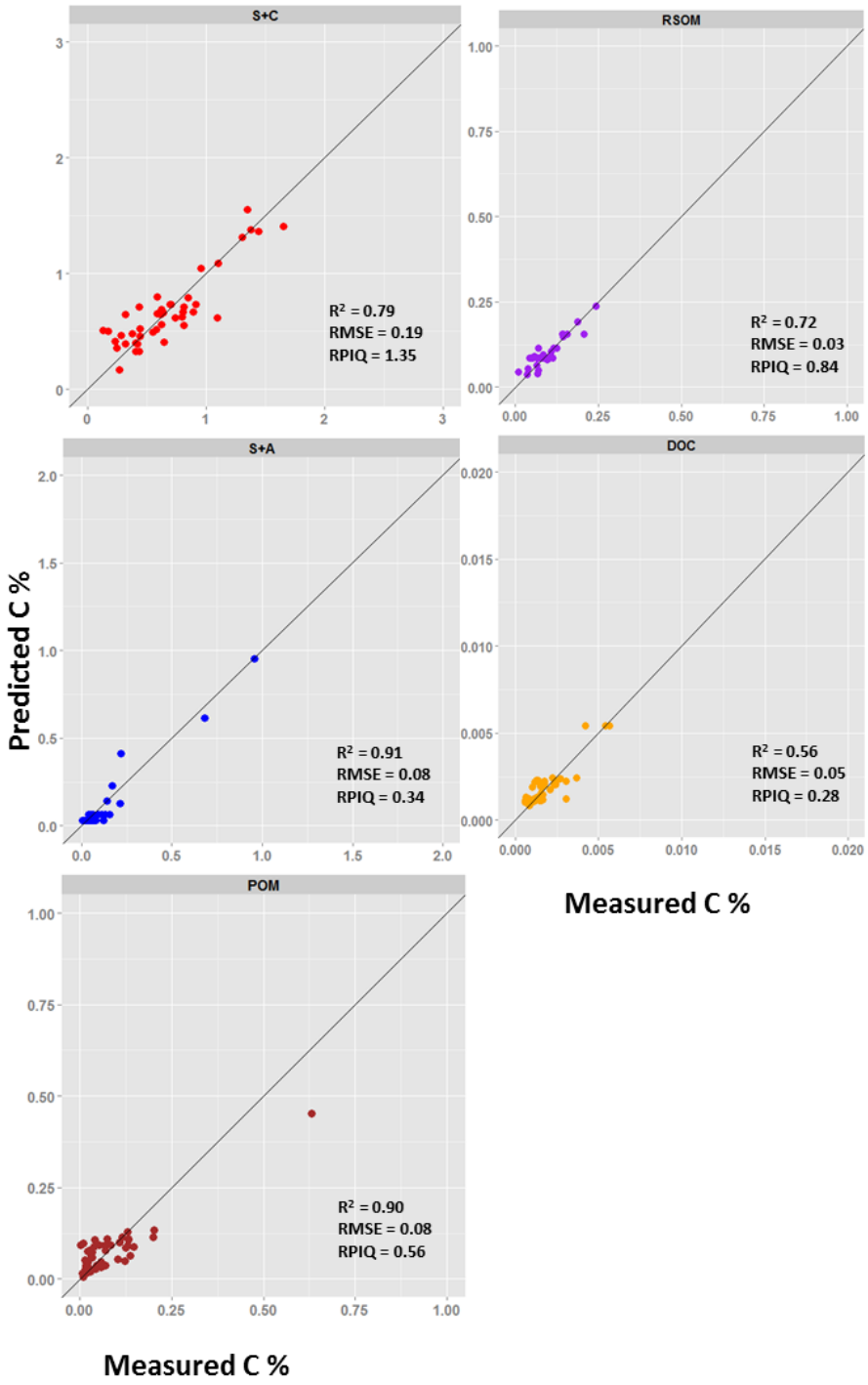


Figure 4.4. Relationship between measured and predicted carbon content of SOC fractions of bulk soil samples.

4.3.4. Carbon pool distribution among aggregate fractions

The summary of statistics for the C distribution of S+C, S+A, LF-POM, RSOC and DOC in aggregate fractions are presented in **Table 4.1.** The S+C fraction is the most dominant fraction among all three aggregate fractions. The highest mean of S+C was recorded in organo-mineral association fraction followed by microaggregates and macroaggregates. Because, organo-mineral associations are resulting from the association of clay and silt particles together and stabilization of SOM into the mineral surfaces (Christensen, 2001). The LF-POM and S+A were observed highest in the macroaagregate fraction. The highest RSOC content was recorded in organo-mineral associations, and is to be expected as SOC sorption on mineral surfaces has been shown be a major process of carbon stabilization. This results in physical or chemical inaccessibility of SOM for decomposing organisms by the formation of closed environments or strong bonds. This may also be the consequence of encapsulation and/or shielding of organic matter from microbial and enzymatic attack (Baldock and Skjemstad, 2000; Krull et al., 2003). Therefore, organo-mineral fraction has shown more RSOC than the other two aggregate fractions.

Table 4.1. Summary statistics obtained for the distribution of SOC fractions in aggregate fractions.

Fraction	Mean (%)	Std. Dev (%)	Maximum (%)	Minimum (%)
Macroaggregates				
S+C	0.28	0.31	1.45	0.011
S+A	0.16	0.13	0.54	0.006
LF-POM	0.26	0.38	2.37	0.006
RSOC	0.05	0.05	0.29	0.004
DOC	0.004	0.003	0.06	0.001
Microaggregates				
S+C	0.32	0.41	1.65	0.03
S+A	0.04	0.04	0.16	0.004
LF-POM	0.07	0.08	0.40	0.005
RSOC	0.07	0.05	0.22	0.013
DOC	0.003	0.002	0.08	0.002
Organo-minerals associations				
S+C	0.54	0.42	1.71	0.07
RSOC	0.16	0.11	0.53	0.05
DOC	0.005	0.003	0.01	0.001

4.3.5. Relationships between carbon pools and the total carbon of aggregate fractions

Figure 4.5. illustrates the relationships between TOC of aggregate fractions and SOC fractions within soil aggregates. LF-POM has shown positive correlations with the TOC of macroaggregate and microaggregate fractions of $\sqrt{0.42}$ and $\sqrt{0.28}$ respectively. Previous studies have shown that POM improves soil aggregation since it can form an organic core surrounded by clay, silt particles, and aggregates (Jastrow and Miller, 1998). LF-POM of macroaggregate fraction was highly correlated with macroaggregated-TOC. Also, bulk soil LF-POM and S+A fractions were highly correlated (R^2 = 0.83 and R^2 = 0.69) with LF-POM and S+A in the macroaggregate fraction (**Figure 4.6**). Composition of free POM in the soil is characterized by a high content of polysaccharides, typically found in fresh plant materials and fungal hyphae and spores (Gregorich et al., 1995; Waters and Oades, 1991). Cambardella and Elliott (1992, 1993, 1994) found that most of this labile pools located within macroaggregate fraction. They suggested these labile SOM are either LF-POM or relatively low-density, mineral-associated OM, probably of microbial origin. This free LF-POM induces the formation of macroaggregates because it is a C source for microbial activity and the production of microbial-derived binding agents (Jastrow, 1996; Golchin et al., 1994; Six et al., 1999). Thus, TOC of the macroaggregete is significantly affected by the LF and S+A.

TOC of organo-mineral associations was positively correlated ($\sqrt{0.30}$) with S+C fraction. Thus, S+C fraction was significantly affected to the TOC of the organo-minerals associations (**Table 4.1)**. The carbon content of the bulk S+C was observed better correlation with the S+C of the organo-minerals associations (**Figure 4.6**). Thus, particle size of the S+C and organo-mineral associations are in similar size (< 53 µm) and the fractionation procedure use.

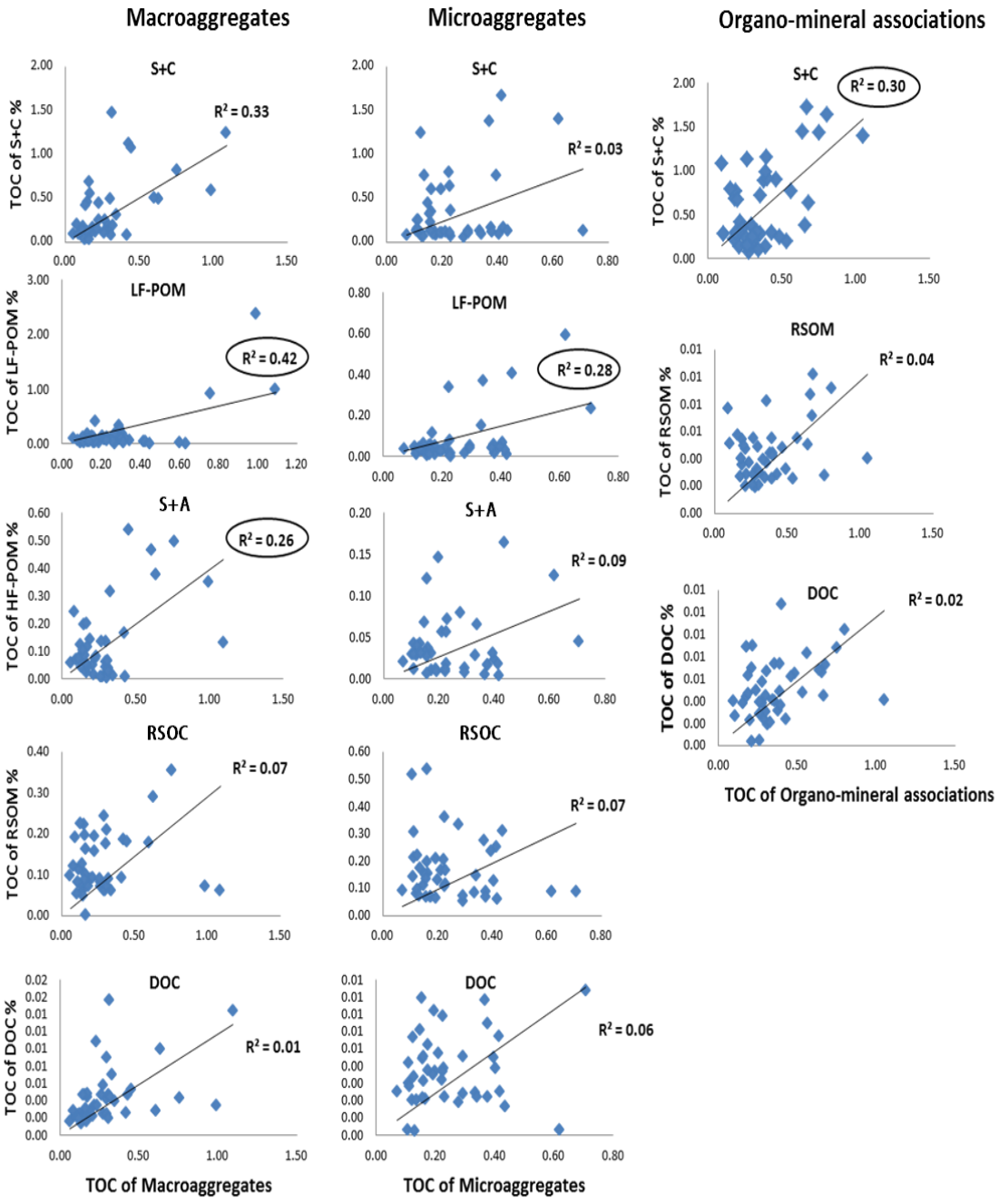


Figure 4.5. Relationships between soil carbon fractions and TOC of macroaggregates microaggregates and organo-mineral associations.

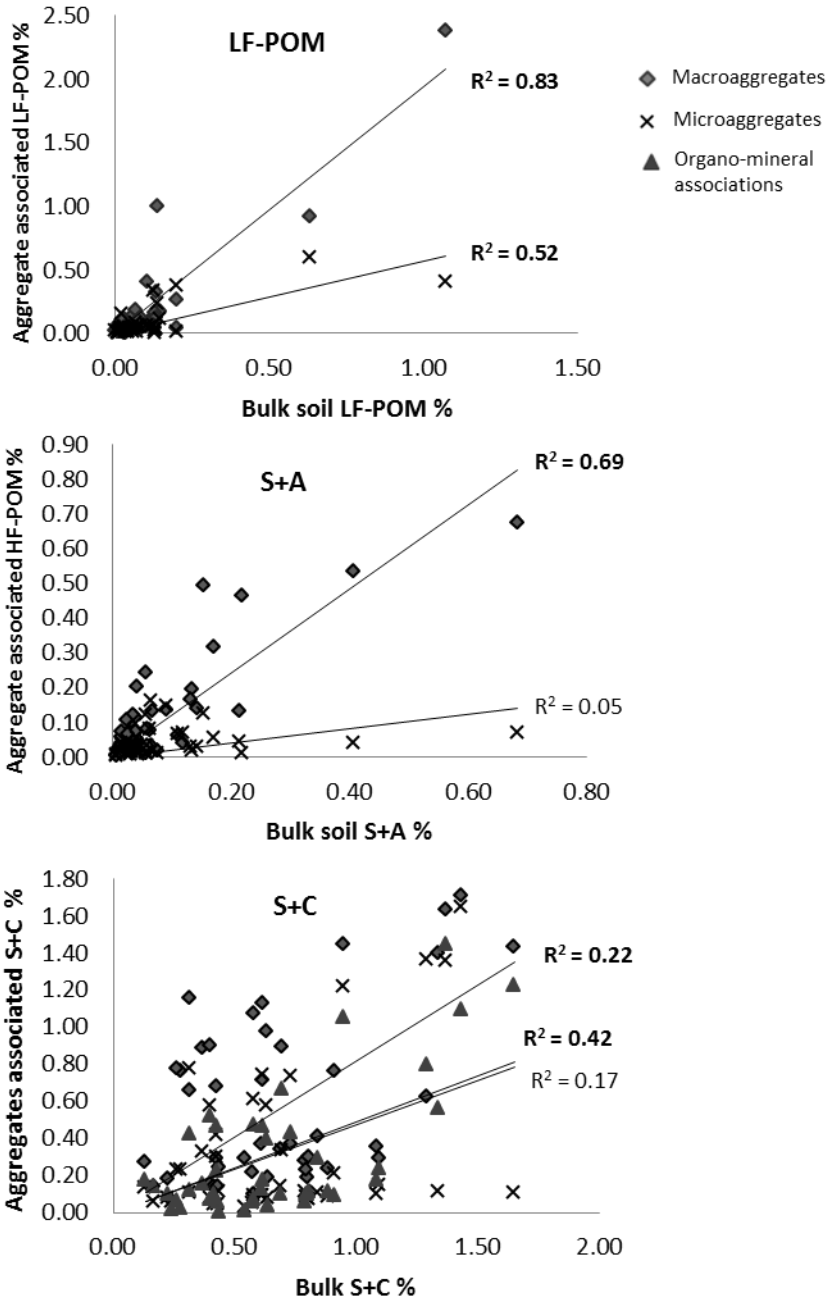


Figure 4.6. Relationships of SOC fractions (S+C, LF-POM and S+A) associated with bulk soils and macroaggaregate, microaggregate and organo-mineral associations.

RSOC and DOC have not shown any positive relationship between TOC of aggregated carbon and bulk SOC fractions. According to the previous studies, RSOC could be expected in better correlation with microaggregate and organo-mineral associated carbon as these fractions were showed high carbon turnover rates compared to macroaggregate associated carbon (Jastrow at al., 1996).

4.3.6. Prediction of carbon pools in aggregate fractions by bulk soil spectra

Table 4.2. contains the performance of the cross-validation results of carbon fractions in aggregate fractions by using bulk soil MIR spectra. The prediction results for LF-POM were excellent in both the macroaggregate and microaggregate fractions. The R^2 for LF-POM was 0.93 with RMSE = 0.08 and for microaggregate fraction 0.91 with RMSE = 0.04. There is clearly a potential to predict LF-POM in aggregate fractions using bulk soil MIR spectra. This is a great advantage as this fraction is the most studied carbon fraction in relation to soil aggregate related research. S+A revealed good prediction result for aggregate fractions. However, the prediction of this fraction was more accurate in microaggregates than for macroaggregates (R^2 = 0.82, RMSE = 0.02) with the highest RPIQ (0.99) among fractions. This fraction consists of sand+stable aggregates. The carbon associated with these aggregates mainly come from the microaggregates and not the macroaggregates, because in this study we used a high level of energy (22 Jml^{-1}) to disperse the aggregates. All the macroaggregates are expected to be disperse with this much energy and the remaining aggregates were most probably microaggregates. Thus, aggregate stability of the microaggregate was higher than for the macroaggregates.

The S+C fractions were found to be quantitatively well predicted in macroaggregate and microaggregate fractions. However, the prediction results for this fraction revealed high RMSE values when compared with other SOC fractions. RSOC showed better predictions only for macroaggregate fraction (R^2 = 073, RMSE = 0.03). DOC predictions showed very poor results for all aggregate fractions. We observed the same prediction patterns for RSOC and DOC fractions in bulk soils. DOC acts as an energy source for

microbes in SOM decomposition (McDowell et al., 2006) and does not show direct importance in aggregate relates studies. However, RSOC is an important fraction in terms of soil carbon stabilization through physical protection of SOM in soil aggregates and biochemical stabilization with mineral associations (Krull et al, 2003). Zimmermann et al. (2007b) also reported poor prediction results for RSOC fraction However, we believe that the oxidation procedure was accurate when compared with the TOC% of S+C and RSOC fractions in macroaggregates. Previous literatures proved NaOCl to be able to oxidize 77% and 95% of the initial SOC (Kaiser and Guggenberger, 2003; Mikutta et al., 2005). Thus, our results showed that NaOCl was able to oxidize most of the macroaggregate fractions' labile SOM, but not microaggregate and organo-mineral associated labile pools. Further, the RSOC pool is stabilized by various mechanisms, and there is still no reliable method to quantify the RSOC (Wiesmeier et al., 2014). Poeplau et al. (2013) reported that the fractionation approach developed of Zimmermannet al. (2007b) has a high unexplained variability when determining the RSOC using NaOCl oxidation. Therefore, the use of other methods such as high energy UV photooxidation might be beneficial for oxidize this highly recalcitrant carbon fractions specially in microaggregate and organo-mineral associations. Skjemstad et al. (1993) demonstrated most of recalcitrant carbon e.g. proteins, lignin, humic acid and alkyl-C were destroyed by direct exposure to the UV photo-oxidation process. Baldock et al. (2013) reported better predictive MIR/PLSR results for bulk soil RSOC with R^2 = 0.85, RMSE = 0.32 using high energy photo oxidation method.

Table 4.2. Summary statistics calculated for MIR-Cubist model derived by using bulk soil spectra to predict S+C, S+A, LF-POM, RSOC and DOC in aggregate fractions.

Carbon pool	Derivatives	RMSE	R^2	RPIQ
Macroaggregates				
S+C	None	0.28	0.74	0.40
S+A	None	0.08	0.68	0.43
LF-POM	None	0.08	0.93	0.52
RSOC	First	0.03	0.73	0.57
DOC	First	0.17	0.73	0.65
Microaggregates				
S+C	None	0.36	0.69	0.13
S+A	First	0.02	0.82	0.99
LF-POM	None	0.04	0.91	0.58
RSOC	First	0.05	0.36	0.32
DOC	First	0.01	0.35	0.79
Organo-mineral associations				
S+C	None	0.52	0.52	0.44
RSOC	None	0.13	0.29	0.35
DOC	None	0.21	0.22	0.48

Table .4.3. Summary table for the Cubist/MIR predictions of the SOC fractions of the aggregate fractions

Fraction	Macroaggregates	Microaggregates	Orgao-mineral associations
S+C	Good	Poor	Poor
HF-POM	Good	Excellent	-
LF-POM	Excellent	Good	-
RSOM	Good	Poor	Poor
DOC	Good	Poor	Poor

4.4. Conclusions

The objective of this study was to examine the potential use of bulk soil MIR spectra to predict SOC fractions in aggregate fractions. Based on the cross validation results for LF-POM and S+A fractions were predicted well in both macroaggregate and microaggregate fractions The prediction of S+C performed well in macroaggregate fractions and slightly less in the other two fractions. Microaggregate and organo-mineral associated DOC predictions were poorer than predictions for other carbon pools. All the carbon fraction predictions related to organo-mineral fractions showed comparably low potentials to use with MIR spectroscopy. This was supported by the **Chapter 3** cross-validation of this fraction , as this also showed low prediction results Also, it is better to use other available fractionation procedures to separate the RSOC fraction as it's an important fraction in both carbon and aggregate turnover models. Considering the satisfactory results obtained from this study, there is a great potential in the use of spectroscopy techniques to measure both chemically and physically protected carbon pools avoiding time and costs of conventional laboratory fractionation procedures.

4.5. References

Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma. 92, 73-85.

Baldock, J.A., Hawke, B., Sanderman, J., Macdonald, L.M., 2013. Predicting contents of carbon and its component fractions in Australian soils from diffuse reflectance mid-infrared spectra. Soil Research. 51, 577-595.

Baldock, J.A., Skjemstad J.O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry 31, 697-710.

Baldock, J.A., Skjemstad, J.O., 1999. Soil organic carbon/soil organic matter. In: Peverill, K.I., Sparrow, L.A., Reuter, D.J., (Eds.), Soil Analysis: An Interpretation Manual. CSIRO Publishing. pp159-170.

Cambardella, C.A., Elliott, E.T., 1993. Methods for physical separation and characterization of soil organic matter fractions. Geoderma. 56, 449-457.

Cambardella, C.A., Elliott, E.T., 1994. Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Science Society of America Journal. 58, 123-130.

Camberdella., C.A., Elliott, E.T., 1992. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Science Society of America Journal. 56, 777-783.

Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science. 52, 345-353.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy. Australian Journal of Soil Research. 32, 285-309.

Gregorich, E.G., Ellert, B.H., Monreal, C.M. 1995. Turnover and storage of corn residue carbon in a Gleysolic soil in eastern Canada. Canadian Journal of Soil Science. 75, 161-167.

Gryze, D. Six, J., Merckyx, R., 2006. Quantifying water-stable soil aggregate turnover and its implication for soil organic matter dynamics in a model study. European Journal of Soil Science. 57, 693-707.

Guggenberger, G., Zech, W., Schulten, H.R., 1994. Formation and mobilization pathways of dissolved organic matter: evidence from chemical structural studies of organic matter fractions in acid forest floor solution. Organic Geochemistry. 21, 51-66.

Janik, L.J., Merry, R.H. Skjemstad, J.O., 1998. Can mid infrared diffuse reflectance analysis replace soil extractions?. Australian Journal of Experimental Agriculture. 38, 681-696.

Jastrow, J.D., Miller, R.M., 1998. Soil aggregate stabilization and carbon sequestration: feedbacks through organo-mineral associations. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A., (Eds.), Soil Processes and the Carbon Cycle. CRC Press, Boca Raton. pp207-223.

Jastrow, J.D., Boutton, T.W., Miller, R.M. 1996. Carbon dynamics of aggregate associated organic matter estimated by Carbon-13 natural abundance. Soil Science Society of America Journal. 60, 801-897.

Jenkinson, D.S., Andrew, S.P.S., Lynch, J.M., Goss, M.J., Tinker, P.B. 1990. The turnover of organic carbon and nitrogen in soil. Philosophical Transactions: Biological Sciences. 329, 361-368.

Krull, E. S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Functional Plant Biology. 30, 207-222.

Malamoud, K., McBratney, A.B., Minasny, B., Field, D.J., 2009. Modelling how carbon affects soil structure. Geoderma. 149, 19-26.

McCarty, G.W., Reeves, J.B., 2006. Comparison of IR and MIR diffuse reflectance spectroscopy for field-scale measurement of soil fertility parameters. Soil Science. 171, 94-102.

McDowell, G.J., Likens, G.E., 1988. Origin, composition and flux of dissolved organic carbon in the Hubbard Brook Valley. Ecological Monographs. 58, 177-195.

Mikutta, R., Kleber, M., Kaiser, K., R. Jahn, R., 2005. Review: organic matter removal from soils using hydrogen peroxide, sodium hypochlorite and disodium peroxodisulfate. Soil Science Society of America Journal. 69, 120-135.

Minasny, B., McBratney, A., Stockmann, U., Hong, S.Y., 2013. Cubist, a regression rule approach for use in calibration of NIR spectra. - 16th International Conference on Near Infrared Spectroscopy. 2 - 7 June 2013.

Noon, C., Stevens, A., Oost, K.V., Six, J., Barthès, B., van Wesemael, B., 2014. Mid-infrared spectroscopy as a tool to identify and quantify soil organic carbon size fractions. Geophysical Research. 16.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in great plains grasslands1. Soil Science Society of America Journal. 51, 1173-1179.

Poeplau, C., Don, A., 2013. Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma.192, 189-201.

Reeves III, J.B., Smith, D.B., 2009. The potential of mid- and near-infrared diffuse reflectance spectroscopy for determining major- and trace-element concentrations in soils from a geochemical survey of North America. Applied Geochemistry. 24, 1472-1481.

Sanderman, J., Baldock, J., Hawke, B., Macdonald, L., Massis-Puccini, A., Szarvas, S., 2011. National soil carbon research programme: Field and laboratory methodologies, CSIRO.

Segoli, M., De Gryze, S., Dou, F., Lee, L., Post, W.M., Denef, K., Six, J., 2013. AggModel: a soil organic matter model with measurable pools for use in incubation studies. Ecological Modelling. 263, 1-9.

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal. 63, 1350-1358.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil. 241, 155-176.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002b. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil. 241, 155-176.

Skjemstad, J.O., Spouncer, L.R., Cowie, B., Swift, R.S., 2004. Calibration of the Rothamsted organic carbon turnover model (RothC ver. 26.3), using measurable soil organic carbon pools. Soil Research. 42, 79-88.

Sohi, S.P., Mahieu, N, Arah, J.R.M., Powlson, D.S., Madari, B., Gaunt, J.L., 2001. A procedure for isolating soil organic matter fractions suitable for modeling. Soil Science Society of America Journal. 65, 1121-1128.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science. 33, 141-163.

Viscarra Rossel, R.A., Walvoort, D.J.J., McBratney, A.B., Janik, L.J., Skjemstad, J.O., 2006. Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. Geoderma. 131, 59-75.

von Lützow, M.,J. Leifeld, Kainz, M., Kögel-Knabner, I. Munch, J.C., 2002. Indications for soil organic matter quality in soils under different management. Geoderma. 105, 243-258.

Waters, A.G., Oades, J.M., 1991. Organic matter in water stable aggregates. In Wilson, W.S., (Eds.), Advances in Soil Organic Matter Research: the Impacts on Agriculture and the Environment. The Royal Society of Chemistry, Melksham, Wiltshire, U.K. pp163-174.

Wiesmeier M., Schada, P., von Lützowa, M., Poeplaub, C., Spörleinc, P., Geuc, U., Hangenc, H., Reischlc, A., Schillingc, B., KögelKnabnera, I., 2014.

Quantification of functional soil organic carbon pools for major soil units and land uses in southeast Germany (Bavaria) Agriculture, Ecosystems and Environment. 185, 208-220.

Williams, P., 2003. Near-infrared Technology-Getting the Best out of Light. PDK Projects, Nanaimo, Canada.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J., 2007a. Measured soil organic matter fractions can be related to pools in the Roth-C model. European Journal of Soil Science. 58, 658-667.

Zimmermann, M., Leifeld, J., Fuhrer, J., 2007b. Quantifying soil organic carbon fractions by infrared-spectroscopy. Soil Biology and Biochemistry. 39, 224-231.

Chapter 5: Application of MIR spectroscopy to estimate the changes of the aggregates carbon and soil organic carbon fractions in incubated soils

5.1. Introduction

The stability of SOC is affected by the land use type (Poeplau et al. 2013), and it was demonstrated that grassland and forest soils have comparatively larger SOC stocks compared to cropland soils (Wiesmeier et al., 2014; Yamashita et al., 2006). The lack of protection of SOC in cropland soil is mediated by the disruption of aggregates due to cultivation practices, such as tillage (Six et al., 2000). In cropland soils around 90% of total SOC stocks exist in intermediate and passive pools, whereas forest soils are dominated by SOC in the active pool (pools as defined in **Chapter 2**). According to Wiesmeier et al. (2014), moisture is the main environmental factor affecting the persistence of SOC in the intermediate pool in cropped soils, while a combination of temperature and precipitation strongly determinates active and intermediate pools in forest soils. Therefore, land use and environmental variables strongly influence SOC dynamics and aggregates that interact with this carbon, which means they are required attributes in developing SOC models. Currently, many SOC models considered a variety of variables other than SOC, for example, soil moisture, soil temperature, plant biomass production, land use systems and so on (Lawrence et al., 2009).

Widely applied SOC models such as Roth-C, Century, Candy etc. are comprised of kinetic relationships quantifying carbon decay and associated biophysical variables as texture, bulk density (particle packing), temperature, and moisture (Lawrence et al., 2009). Even though bulk density is included this does not describe where and how the SOC interacts with the mineral phase. However, there are research gaps in both physical SOC protection mechanisms under different land-use systems and diverse environmental conditions. The most recent research, for example Wiesmeier et al. (2014) and Poeplau

et al. (2013), still focus on the chemical breakdown of separated SOC fractions of soils under cropland, grassland and forest land use systems, and this research affirms the effect of temperature and moisture on decay rates of SOC fractions in different land use systems. Similar studies were conducted by Yamashita et al. (2006) and Johna et al. (2005), where the effect of land use on storage of SOC fractions in aggregate fractions was only investigated. However, none of these studies considered the role of aggregates affecting the decay of the SOC even after the acceptance of these relationships originally described by Emerson (1959), Tisdall and Oades (1982), and Golchin et al. (1994).

To combine findings of SOC-aggregate relationship and SOC turnover models research is required to understand both the chemical and physical protection of soil carbon pools. It is important to evaluate the dynamics these pools under different land use systems from rapid microbial decomposition with a range of environmental factors (Creamer et al., 2012; Gu et al., 2004; Haile-Mariam et al., 2008). Therefore, in this chapter was focused to use MIR spectroscopy to quantify changes in soil aggregated-C and SOC fractions of incubated soils under different land use systems at different temperature levels. The main objectives of this study are

1. To quantify the distribution of SOC fractions and aggregate fractions under different land use systems.
2. To determine the effect of temperature on physically and chemically protected SOC fractions over time.
3. To identified spectral differences in incubated soils and correlated these changes to turnover of SOC fractions.
4. To use MIR/Cubist models developed in the Chapter 4 to predict changes in SOC fractions after the incubation period.
5.

5.2. Materials and Methods

5.2.1. Study area

The study area was located around Pokolbin in the lower Hunter Valley in New South Wales, Australia. This region has a relatively long wine producing history, first established in the 1840s (Loughran et al., 2000). The study area covered approximately 279 km^2 is cantered on 151.31°E, 32.76°S. The climate of the area is temperate, with the maximum daily mean annual temperature ranging from 5 ^{0}C to 30 ^{0}C. The mean annual precipitation is ranging from 300-1000 mm.

The elevation of the area is between 25-120 m in the low relief area and increases to around 540 m in the high relief area. The soils in the study area are dominated with red Dermosols and flowed by Brown, Black and Grey Dermosols. The predominant land use across the study area consists of native pasture, native forest and irrigated viticulture.

5.2.2. Soil sampling

Sampling sites were selected based on soil texture, soil orders and land use types of Hunter Valley area (**Figure 5.1**). In order to compare similar soils under different land uses Red Dermosols were selected as the studied soil sub order. Soils were selected from vineyard, forest and unimproved pasture lands from 0-30 cm depth with 10-15 % of clay. Sampling was done randomly from five locations where these three land use types were located adjacent to each other.

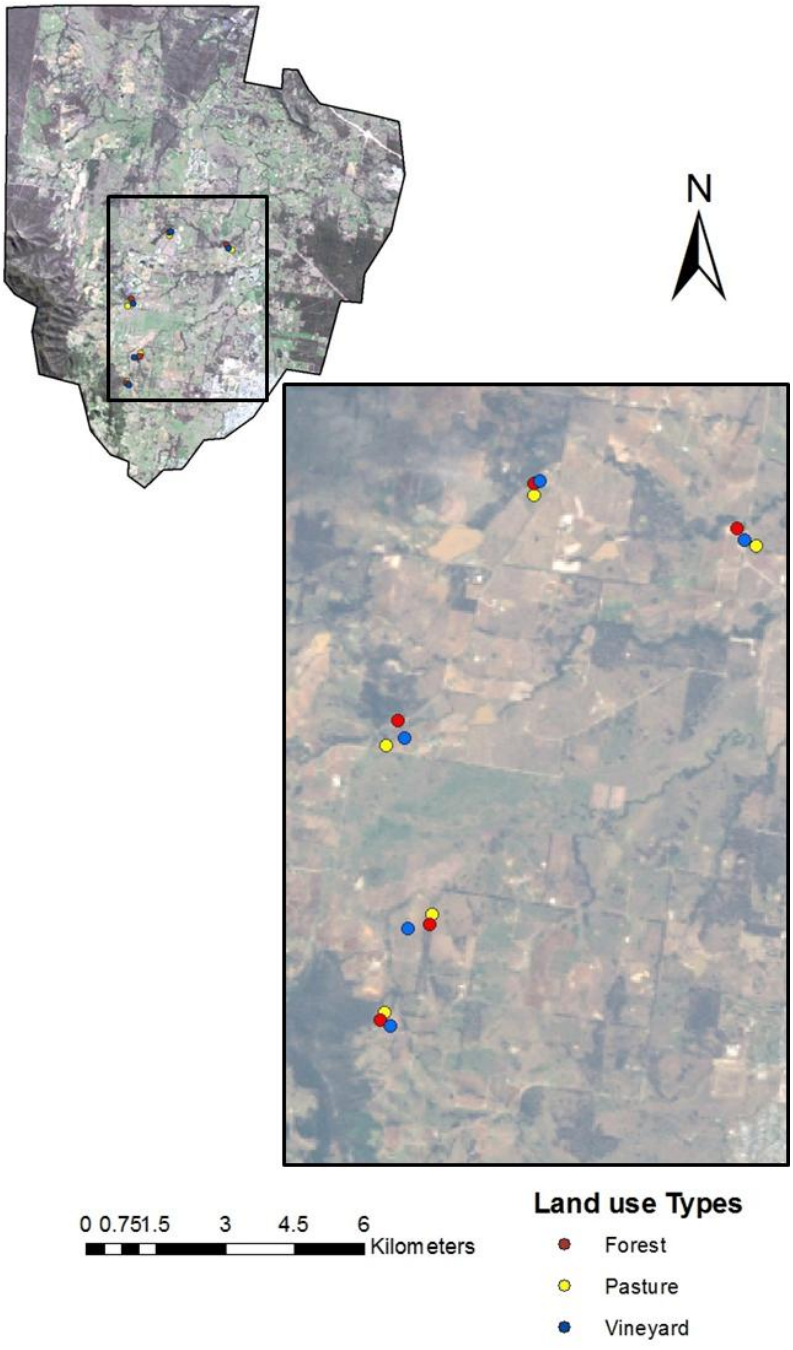


Figure 5.1. Study area and sampling locations of Hunter Valley

5.2.3. Initial soil analysis

Soils were analyzed for soil moisture, pH, texture, microbial biomass carbon (MBC), and water holding capacity (WHC), TOC content, aggregate size distribution, aggregate associated-C and SOC fractions

Soil pH was measured at a ratio of 1:5 (w/w) of soil to water, using a pH meter (White, 1969). Soil texture was determined using hydrometer method (Gee and Bauder, 1986) on a 30 g subsample dispersed with 50 ml of 5% sodium-hexametaphosphate solution for 48 hours.

The chloroform fumigation extraction method (Vance et al., 1987) was used to measure MBC by extracted with 100 ml 0.5 M K_2SO_4 with a conversion factor (kc) of 0.35 (Jenkinson and Ladd, 1981). Water holding capacity was estimated gravimetrically by using 100g of > 2 mm sieved soil, wetted slowly with 8 ml of deionized water in glass test tubes, covered with perforated parafilm, and allowed to equilibrate overnight. A subsample from the middle of the column was then weighed, dried overnight in a 105 ^{0}C oven and weighed again. WHC using the equation:

$$\text{Water holding capacity \%} = \frac{(\text{Wet weight} - \text{Dry weight})}{\text{Dry weight}} \times 100 \quad [5.1]$$

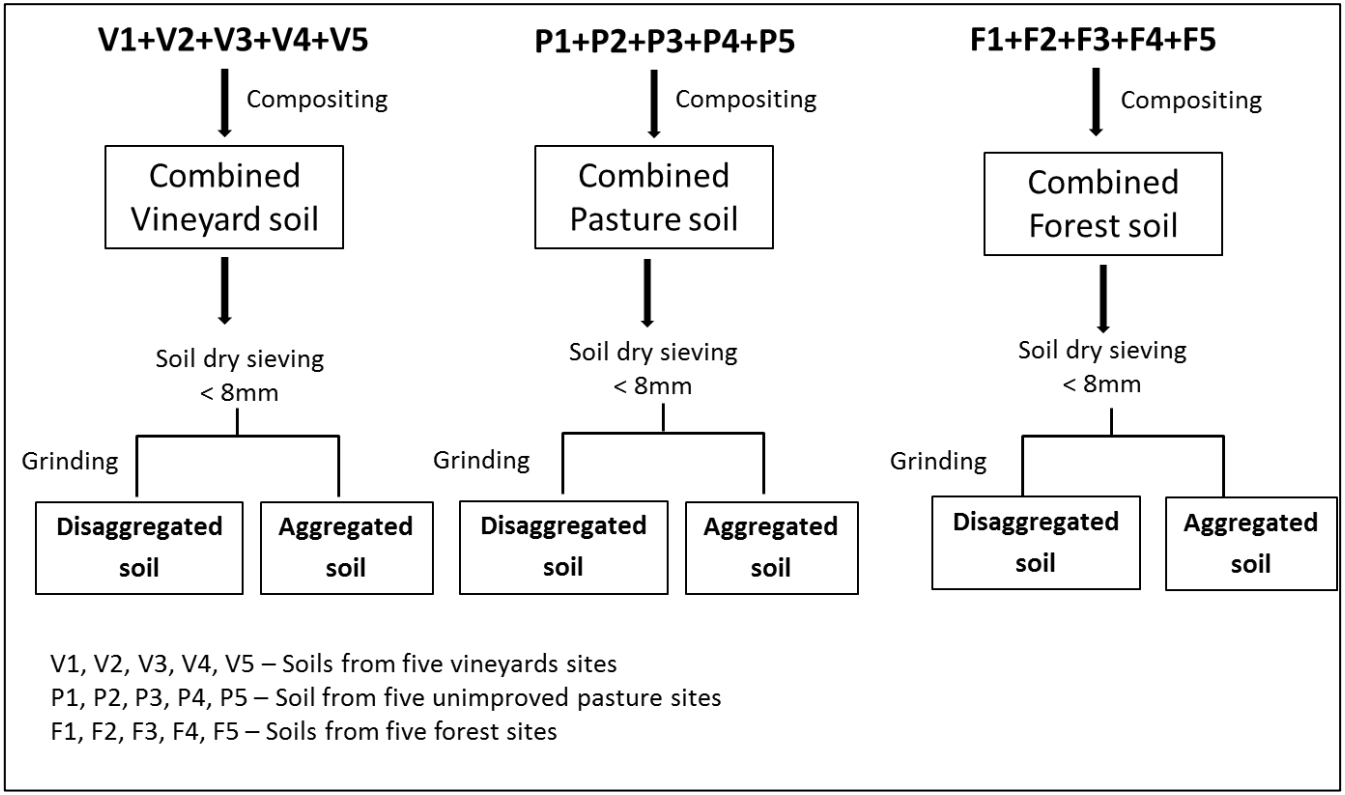


Figure 5.2. Initial soil preparation and soil disaggregation for considered land use types.

Soil aggregate separation and SOC fractionation were done by wet sieving and combined physical and chemical fractionation methods as described in **Chapter 3.2.2** and **Chapter 4.2.1** respectively.

All solid fractions C were measured by dry combustion using Vario Max CNS analyzer (Elementar Analysen systeme GmbH, Hanau, Germany) and liquid fraction (DOC) were anlysed by Shimadzu TOC-VCSH analyser.

5.2.4. Soil disaggregation and initial soil preparation

The five soil samples of each land use type were composited into one vineyard, one pasture, and one forest soil to obtain a most representative sample for each land use system (**Figure 5.2**). Soils were sieved to 8mm diameter size. Combined soil samples (< 8mm sieved) were ground by using a Pulverisete 2 (Fritsch, Germany) electrical grinder for 10 minutes to disaggregate the soil. Initial soil parameters for the composite soil samples are described in sections **5.2.4**. Aggregated and disaggregated soils and aggregate fractions were ground by using Pulverisete 2 (Fritsch, Germany) for 10 minutes.

5.2.5. Incubation study

5.2.5.1. Experimental setup

Aggregated and disaggregated soils were wetted to maintain 60% WHC. Soils were weighed to 5 g and 175 g and placed in 7 ml and 250 ml containers. 5 g of soil was kept to get the MIR spectra monthly and 175 g of soil used as the main incubation soil mass to harvest at the end of the incubation period. Uniform mass to volume ratio was kept to ensure similar bulk the densities in both 7 ml and 250 ml containers with soils. 50 ml

plastic container were filled with 20 ml of 4 M NaOH to capture the CO_2 evolved. The experiment unit was set up and incubated at 5 ^{0}C, 20 ^{0}C and 45 ^{0}C for 180-days. These three temperatures were selected based on the maximum daily mean annual temperature gradient varied from 7 ^{0}C - 45 ^{0}C at Sydney region. Three replicates were maintained for each treatment. Three control incubation chambers were set up in the same manner without containing soils as a control for to account for the atmospheric CO_2 present in the headspace of the incubation chambers at three temperature levels. Once a month Glucose was applied to soil enhance the microbial activity and incubation units were aerated with an electrical fan for 2 minutes to refresh the O_2 content of the incubation chambers. The NaOH containers were replaced 1, 2, 7, 14, 30, 33, 44, 60, 63, 74, 90, 93, 104, 120, 123, 134, 150, 153, 164 days after the experiment was set up.

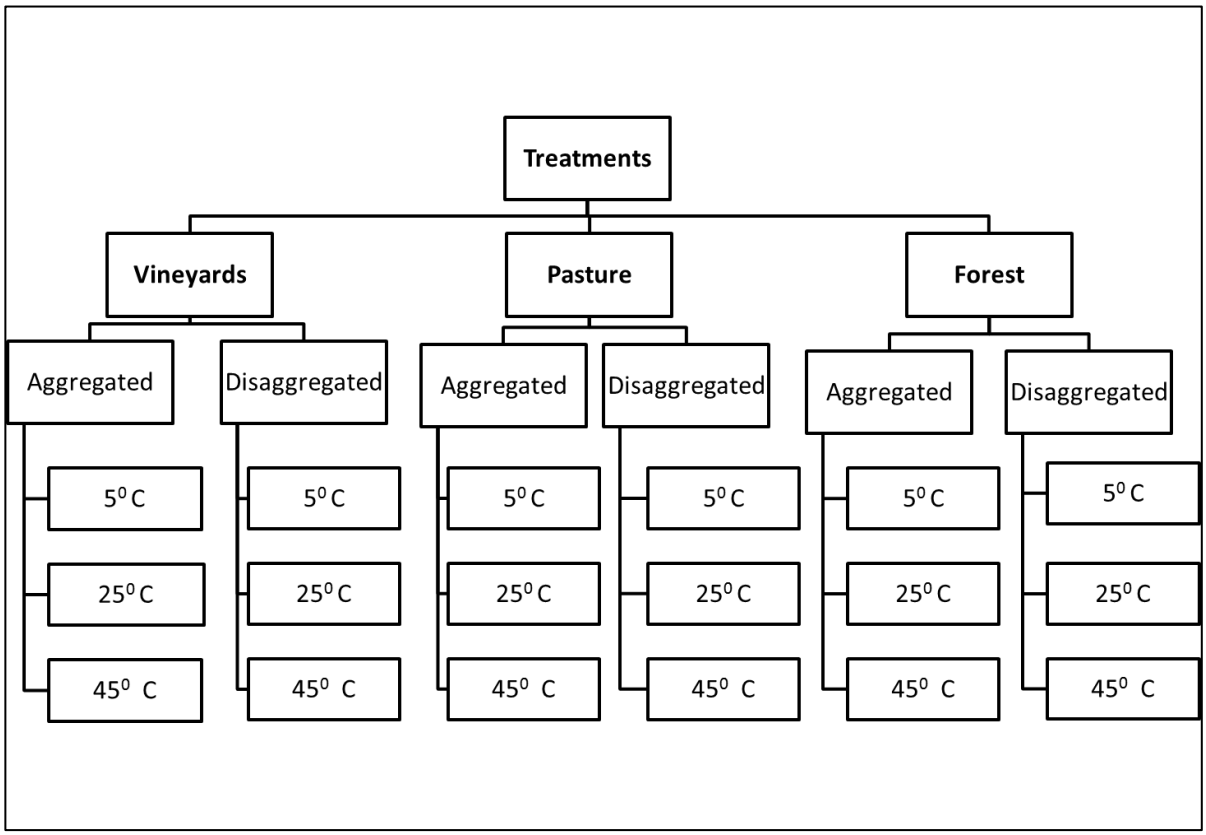


Figure 5.3. Experimental setup for the incubation study

5.2.5.2. Glucose application

Initially and 30, 60, 90, 120, 150 days of incubation glucose was added to the soil to minimize the substrate limitation for microbial soil respiration (Conant et al., 2004; Bradford et al., 2008). The glucose concentration was 12.6 mg ml/1 of glucose solution (0.6 g glucose kg^{-1} soil equivalent to 0.24 g C kg^{-1} soil). The amount of glucose solution added was 0.25 ml/ 5 g to small containers and 8.2 ml/ 175 g of soil in the large containers

5.2.5.3. Sampling and measurements

The small soil containers were collected every month from each experimental unit (7 ml). Soils were ground to < 53 µm and scanned by the Bruker Tensor 37 spectrophotometer. The incubated samples were harvested 180-days after the incubation. Then the soils were oven dried at 40 ^{0}C. Soil moisture, pH, MBC, TOC and aggregate size distribution were analyzed according to the methods described in **section 5.2.4.**

5.2.6. Temperature sensitivity (Q_{10} value)

The temperature sensitivity of TOC mineralization, referred to as Q_{10}, is defined as the rate of increase in soil CO_2 emission with a 10 °C increase in temperature (Kirschbaum, 1995). A first-order exponential equation was used to calculate the Q_{10} values for bulk soil, aggregate fractions and carbon fractions. This equation assumes that Q_{10} is a constant over the temperature gradient (Fang and Moncrieff, 2001).

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{10/(T_2 - T_1)} \quad [5.2]$$

Where, R_2 and R_1 are C depletion rates (% TOC/day) observed at temperatures T_2 (45 ^{0}C) and T_1 (5 ^{0}C), respectively.

5.2.7. Statistical analysis

Statistical analyses were conducted in JMP v. 9 (SAS Institute, Cary, NC, USA) statistical program. A general ANOVA was applied followed by Tukey's HSD test to compare the means of the data obtained at the initial and 180-days after incubation i.e. bulk soil derived TOC, MBC, aggregate mass differences, aggregate-associated C and SOC fractions. The means were evaluated at the 5% significance level unless stated otherwise.

5.2.8. Spectral analysis

Ground samples were scanned by Bruker Tensor 37 spectrophotometer within the spectral range from 2500 to 25000 nm (MIR region) with the method described in **Chapter 3.2.4.1**. The MIR/Cubist models developed to quantify S+C, LF-POM, S+A in bulk soils were used to predict these fractions from the spectra of the incubated soil Zimmerman et al. (2007) revealed a strong correlation of this combined (S+A)+(S+C) fraction and RSOC fractions with humified soil organic matter (HUM) and inert organic matter (IOM) fractions with Roth-C model pools.. Thus, in this study S+A and S+C fractions were combined together (**Figure 5.4**).The spectral pre-processing and model evaluations were completed using the methods described in **Chapter 3.2.5** and **3.2.7.**

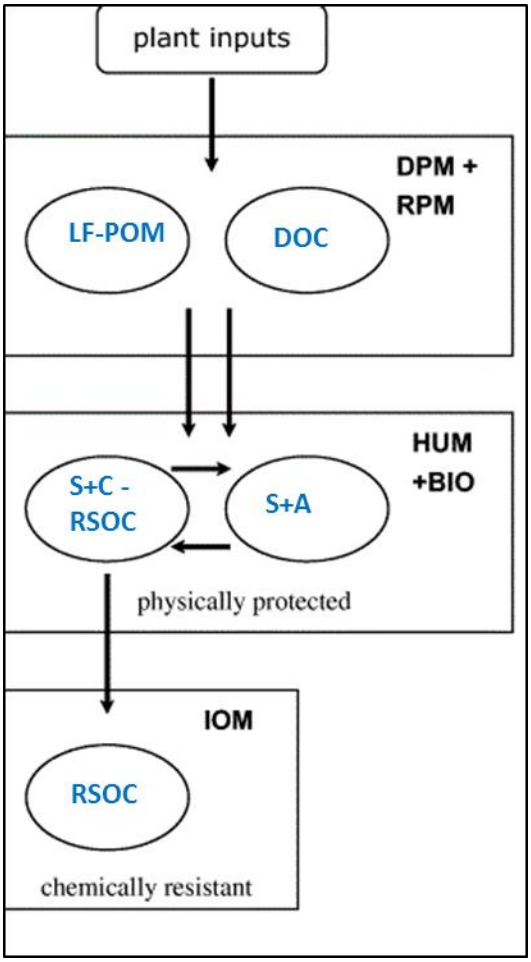


Figure 5.4. Summarizing of SOC pools according to the Roth-C model (adopted from Zimmermann et al., 2007).

5.2.9. Mean differences of absorption spectra

Mean absorptions were calculated using initial soil spectra of the vineyard, pasture and forest soil samples and the spectra collected after 180-days after incubation (DAI). Absorption differences were calculated by the equation below.

$$\text{Absorption difference} = \text{Absorptions of initial soil spectra} - \text{Absorptions of spectra 180 DAI}$$

5.3. Results and Discussion

5.3.1. Initial soil properties

The **Table 5.1** shows the initial soil properties of the vineyard, pasture and forest composited soils. The forest soil has the highest TOC % of the land use systems, while the LF-POM and DOC were comparatively low in the vineyard soils compared to the pasture and forest soils. This was to be expected as many studies have shown LF-POM to be more sensitive to management practices and greatly depleted upon cultivation (Six et al., 2002), and the continuous cultivation of the vineyard soil more than likely contributed to the decrease in the labile pools. The macroaggregated-C was largest in the pasture soils and would be comprised of LF-POM (Cambardella and Elliott, 1994). The smaller quantity of LF-POM faction in the vineyard soil translates to a smaller macroaggregated-C. The observed HUM fraction was larger in vineyard (70.73%) soils than pasture (65.9%) and forest soils (63.4%). This fraction consists of stable aggregates and silt+clay associated-C (Zimmerman et al., 2007). The storage of SOC in silt and clay particles (S+C fraction) is generally larger in cultivated soils compared to forest and pasture soils (Wiesmeier et al., 2014).

Microaggregated and organo-mineral associated-C were highest in vineyard soils. Bongiovanni and Lobartini. (2006) reported microaggregate (250-53μm) and organo-mineral associations were twice as large in cultivated compared to undisturbed soils. The loss of SOC due to macroaggregate disruption in cropland soils enhances the formation of organo-mineral associations as a result of tillage that continuously increases the contact of crop residues with available mineral surfaces (Wiesmeier et al., 2014). The RSOC fraction was more associated with vineyard soils than forest and pasture soils. Thus, croplands are more advantageous for long-term SOC storage than forest and pasture soils as the amount of HUM and resistant SOC is higher than in forest and grassland soils.

Table 5.1. Initial soil properties of combined vineyard, pasture and forest soils.

Soil property	**Land use type**		
	Vineyards	**Pasture**	**Forest**
Soil pH	6.44	5.98	5.98
Clay %	13.33	10.02	9.81
Silt %	20.01	30.31	32.11
Bulk soil TOC (%)	1.64	3.82	4.03
Macroaggregate C (%)	0.93 (56.7)	2.96 (77.7)	2.97(73.7)
Microaggregate C (%)	0.35 (21.3)	0.55 (14.40)	0.70 (17.3)
Organo-mineral associated-C (%)	0.29 (17.7)	0.41 (10.7)	0.40 (9.9)
Microbial biomass C (µg/g)	193.52	284.32	475.44
HUM (%)	1.16 (70.73)	2.51(65.70)	2.56 (63.52)
LF-POM (%)	0.13 (8)	1.01 (26.5)	0.99 (24.5)
RSOC (%)	0.23 (14)	0.42 (11)	0.41 (10.2)
DOC (%)	0.01(0.6)	0.06 (1.6)	0.03 (0.74)

Note: Proportion of C of fraction to bulk soil TOC is indicated within the parenthesis.

5.3.2. Effect of glucose application on soil

Glucose is widely used to determine the parameters microbial growth and microbial biomass in soils (Paul and Clark, 1996). Based on previous work (**Table 5.2**) expected the applied glucose associated-C (glucose-C) would be mineralized at end of every 30-days.

According to the glucose mineralization rate observed in those studies, it is reasonable to expect the applied of 0.024 % of glucose-C in the present study to be totally mineralize after 30 days in the temperature range 5-45°C.

Table 5.2. Mineralization of amended glucose-C in different studies.

Reference	Rate of glucose-C applied (%)	Incubation time (days)	Incubated temperature (^{0}C)	Glucose-C mineralized amount (%)
Schneckenberger et al.(2008)	0.02	6	18	38
Saggar et al. (1999)	0.2	3	25	25-44
Nguyen and Henry (2002)	0.002-0.05	6	22	37-53
Sharabi and Bartha (1993)	0.1	30	27	77

Secondly, we looked for the presence of glucose peaks in the spectra obtained from the 180-days incubated vineyard, pasture and forest soils (**Figure 5.5, 5.6 and 5.8**) at different temperature levels. According to the literature, pure glucose peaks are dominant at wavelengths 3393, 6798, 6909, 8881, 10874, 11842 nm (Sivakesava et al., 2001) and we did not observe any of these peaks in the 180-days incubated soils. This confirmed that the glucose-C did not affect the TOC content of the soil at the end of the incubation period.

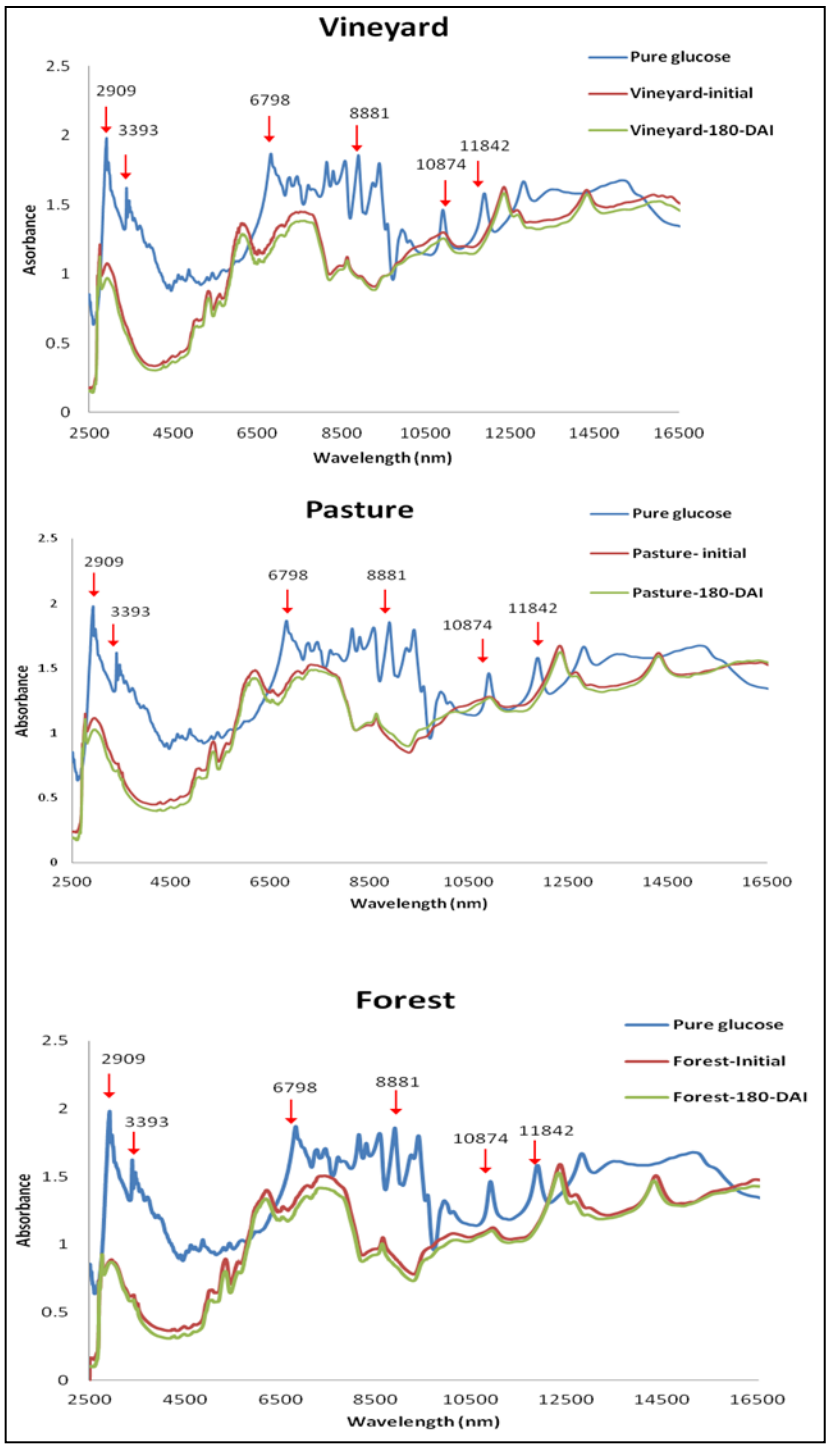


Figure 5.5. MIR spectra of pure glucose, initial and 180-days after incubation (DAI) incubated vineyard, pasture and forest soils at 5 ^{0}C.

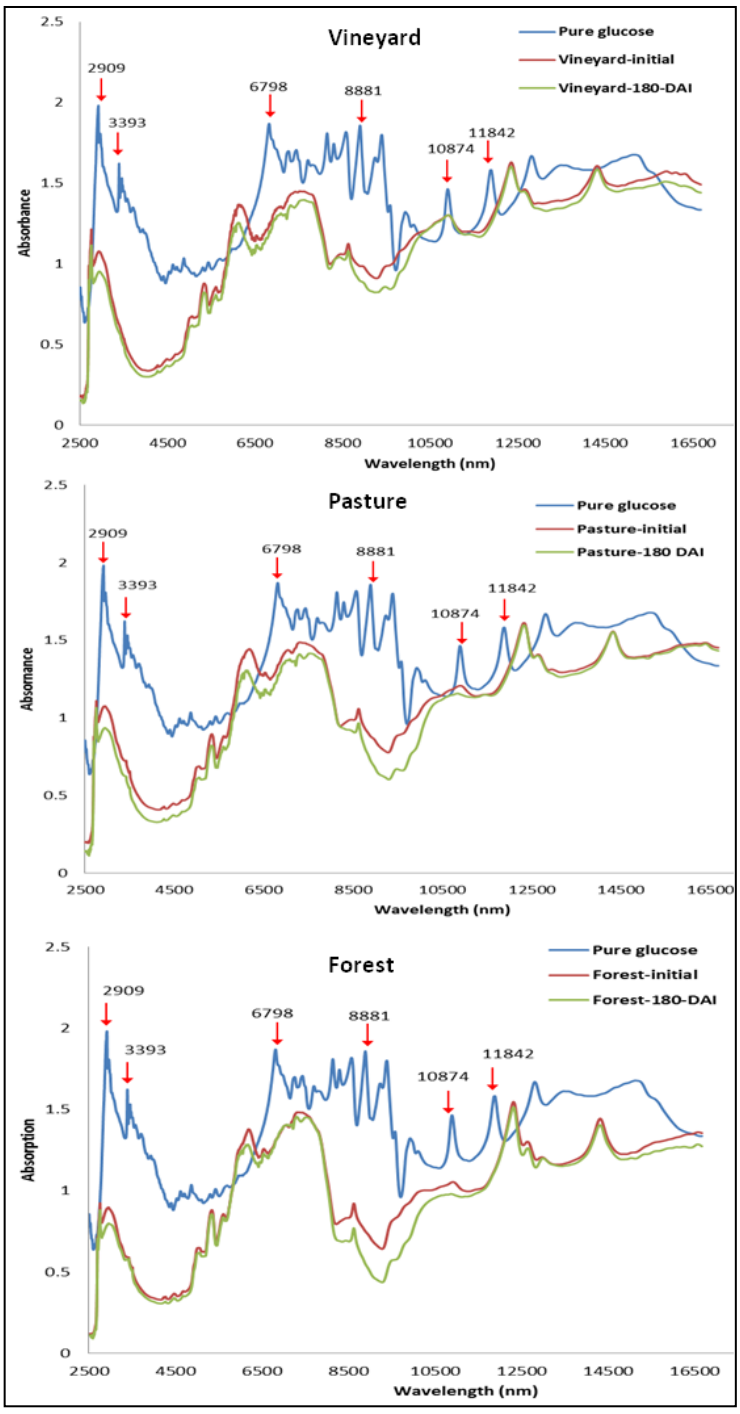


Figure 5.6. MIR spectra of pure glucose, initial and 180-days after incubation (DAI) incubated vineyard, pasture and forest soils at 25 ^{0}C.

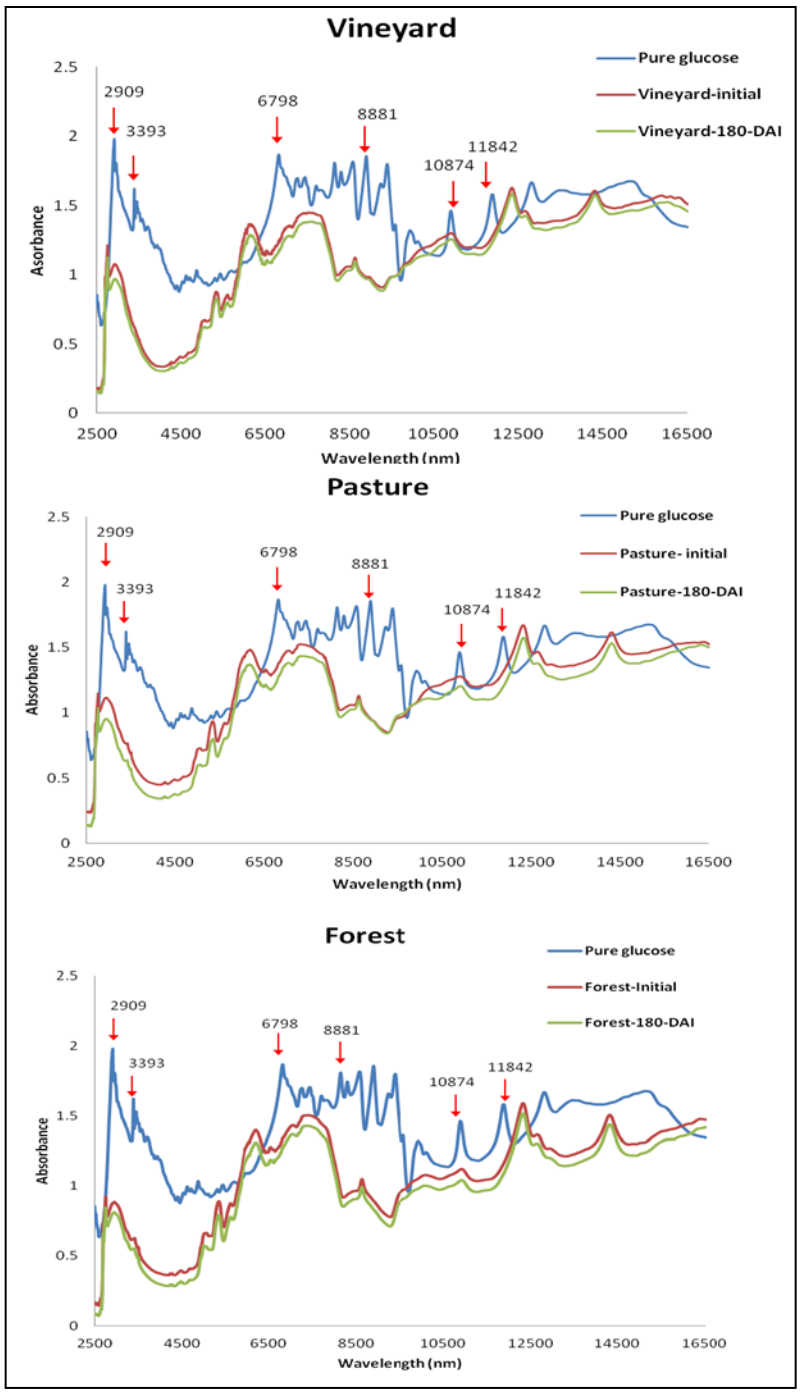


Figure 5.8. MIR spectra of pure glucose, initial and 180-days after incubation (DAI) incubated vineyard, pasture and forest soils at 45 ^{0}C.

Microbial population increased after the incubation period of vineyard, pasture and forest soils (**Figure 5.6**). This increase was greater in disaggregated soil compared to the aggregated soils in forest land systems. This agrees with a previous study showing glucose application rapidly increased the spatial distributions of bacteria and fungi on the surface of soil aggregate but not inside (Chenu and Bloem, 2001). As the surface area of the disaggregated soils is larger and microbes can easily increase their population over the disaggregated soils than the aggregate soils. Under all the land use systems, the microbial population significantly increased at temperature between 5 ^{0}C and 25 ^{0}C and only slightly increased at 45 ^{0}C. This decrease at 45 ^{0}C could be because fungal and bacterial growth rates have shown optimum at temperature around 25-30 ^{0}C and above the optimum level microbial growth is decreasing, but some activity has observed at 45 ^{0}C (Pietikåinen et al., 2005).

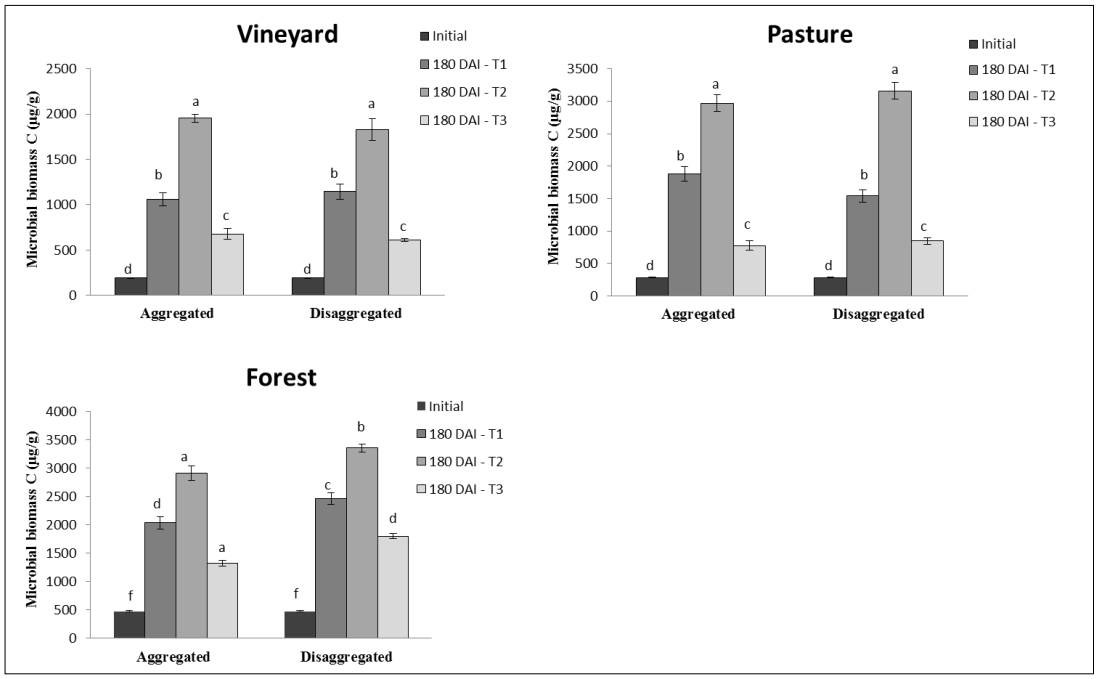


Figure 5.6. Changes of the MBC content initial and 180 days after incubation (DAI) at temperature 5 ^{0}C (T1), 25 ^{0}C (T2) and 45 ^{0}C (T3). Error bars are standard error of means. Different letters denote significant difference at $p < 0.05$.

5.3.3. Changes of the TOC of bulk soil

The measured TOC % of vineyards, pasture and forest soils decreased with increase of the incubation time (**Figure 5.7**).

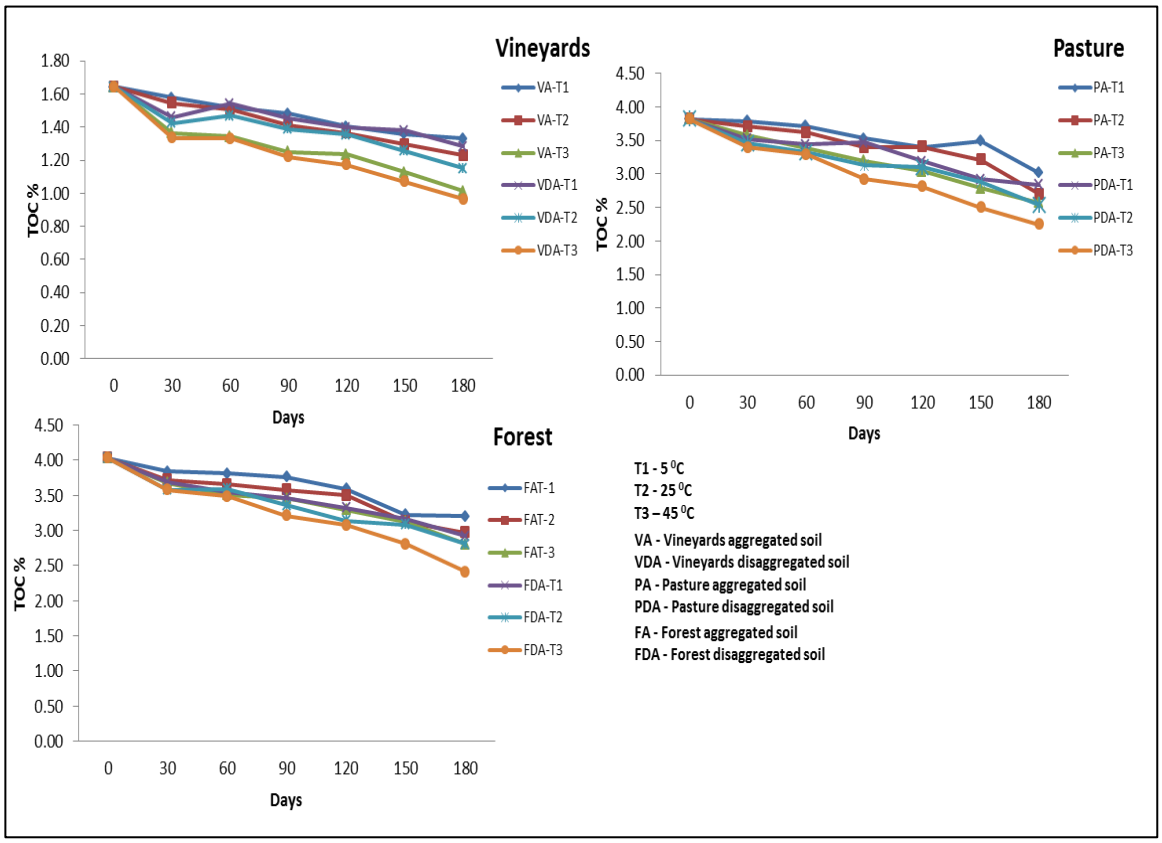


Figure 5.7. Changes of the TOC % of vineyard, pasture and forest incubated soils during the incubation period at temperature 5 ^{0}C (T1), 25 ^{0}C (T2) and 45 ^{0}C (T3).

Table 5.3 shows the amount of TOC decreased after the incubation period compared to initial TOC of the soils. This shows glucose application had a positive priming effect on the SOM decompositions. Land use systems greatly affected the C depletion due to the variations in the distribution and stabilizing mechanisms of the SOC among the land use systems as discussed in **section 5.3.1**. The TOC % of pasture and forest soils decreased significantly with all compared to the vineyards soils. This was mainly due to the presence of larger labile SOC pool in both pastures and forest soils and greater stable

SOC in vineyards soil (**Table 5.1**). Labile C is the fraction has the highest readily available C source for microbes and with most rapid turnover times and this pool provides an early indication of SOM degradation (Olk and Gregorich, 2006; Weil et al., 2003).

Table 5.3. Average mineralized TOC (%) after 180-days of incubation period

Land use type	**Treatment**	**Amount of TOC (%) decreased after the incubation**		
		5 ^{0}C	**25 ^{0}C**	**45 ^{0}C**
Vineyards	Aggregated	11.83^{c}	21.70^{abc}	28.36^{abc}
	Disaggregated	18.21^{bcd}	20.56^{abcd}	33.31^{abc}
Pasture	Aggregated	18.61^{bc}	21.97^{abc}	26.77^{abc}
	Disaggregated	21.53^{abc}	28.20^{abc}	35.71^{abcd}
Forest	Aggregated	20.96^{abc}	23.70^{abc}	27.81^{abc}
	Disaggregated	27.57^{abc}	32.73^{ab}	37.67^{a}

Different letters denote significant difference at $p < 0.05$.

5.3.4. Effect of soil disaggregation on SOC mineralization

Initially, macroaggregates were dominated in all the sites and it was 64, 53 and 48 % of the dry soil weight in forest, pasture and vineyard, respectively. The microaggregate fraction is significantly larger in vineyard soils and these findings are comparable with the previous studies reporting aggregate distribution in different land use systems (Jastrow et al., 1996; John et al., 2005; Six et al., 2000). The grinding treatment

decreased the macroaggregates by 37.12% for pasture soil and 29.98%, 31.74% for forest and vineyard, respectively (**Figure 5.8**). The weight changes after the grinding of microaggregate fraction was not able to clearly differentiate as disrupted macroaagregate soils (< 250 µm) were accumulated in both microaggregate and organo-mineral association fractions.

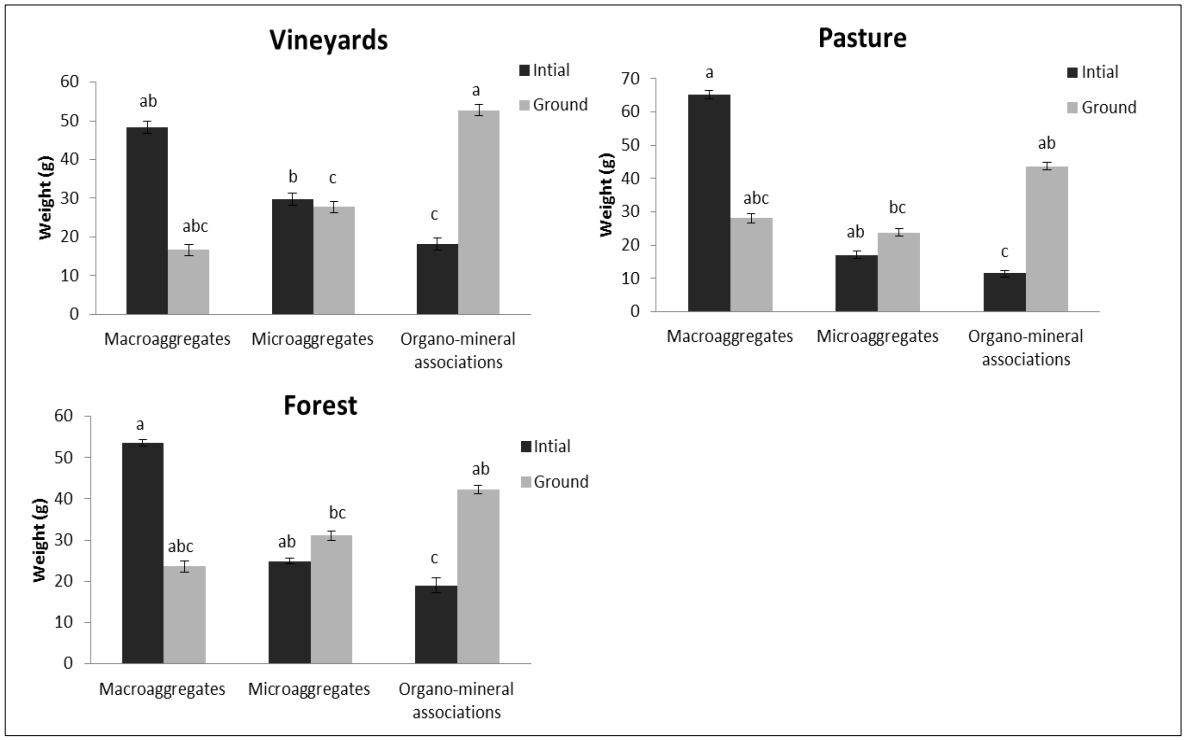


Figure 5.8. Effect of grinding to disaggregate soils in different aggregate fractions of vineyards, pasture and forest soils. Error bars are standard error of means. Different letters denote significant difference at $p < 0.05$.

The disaggregated soils had a larger TOC% depletion (average 7%) compared to the other aggregated soils in all the land use systems (**Table 5.3**). There was an increase in microbial population in disaggregated soils which, in turn, greatly affected to the depletion of more C from the soils. Therefore, soil aggregation has greatly influenced protecting SOC by microbial decomposition. Because SOC inclusion in aggregates which separates the microbes and their enzymes physically from the substrate, limiting

decomposition (Cambardella and Elliott, 1992; Golchin et al. 1994; Six et al., 2002). However, Mueller et al. (2014) observed 43.8% increase of C mineralization of disaggregated soils than aggregated soils as they have used ultrasonic energy to disperse soil aggregates.

5.3.5. Changes of aggregate associated carbon

Table 5.4 shows the changes of C content of aggregate fractions after 180-days incubation at 5 ^{0}C, 25 ^{0}C and 45 ^{0}C temperature levels. The macroaggregate C significantly decreased after 180-days of incubation at all temperature levels. The macroaggregate show a fast turnover rate (31 days) and their breakdown releasing labile SOC (Elliott, 1986; Segoli et al., 2013). Pasture and forest soils lost greater % of C from this fraction than the vineyard soils and this is because more labile SOC (LF-POM and DOC) is observed in pasture and forest soils than vineyard soils, where the labile fraction is more sensitive to microbial utilization. A large portion of the microbial population and enzyme activity is observed in the light fraction (Kanazawa and Filip, 1986). Elliott (1986) reported that macroaggregates contain more labile and less highly processed SOM than microaggregates. Therefore, macroaggregate associated-C significantly decreased during the incubation period. Microaggregate and organo-minerals associated-C depletion was not significantly different at temperature 5 ^{0}C and 25 ^{0}C. However, the temperature of 45 ^{0}C significantly influenced to decrease C from the all the aggregate fractions and even so, this decrease is comparatively low in organo-mineral associated-C. Since, SOC absorbed to this fraction are mainly humified products and highly stable and resistant to temperature changes (Kögel-Knabner et al., 2008).

Table 5.4. The amount of C % decreased from macroaggregates, microaggregates and organo-mineral associations after the incubation period.

Land use system	Aggregate fraction	C %		
		5 ^{0}C	25 ^{0}C	45 ^{0}C
Vineyard	Macroaggregates	0.13(13.47)^a	0.19 (20.32)^a	0.23 (29.20)^a
	Microaggregates	0.02 (6.28)^b	0.04 (9.81)^b	0.04 (13.08)^b
	Organo-mineral associations	0.01 (2.00)^c	0.01 (3.64)^c	0.01 (5.23)^c
Pasture	Macroaggregates	0.64 (21.60)^a	0.75 (25.39)^a	0.87 (29.28)^a
	Microaggregates	0.04 (7.95)^b	0.06 (10.75)^b	0.08 (14.87)^b
	Organo-mineral associations	0.02 (4.73)^c	0.03 (7.23)^c	0.04 (9.69)^c
Forest	Macroaggregates	0.55 (18.67)^a	0.82 (27.75)^a	0.87 (29.18)^a
	Microaggregates	0.04(5.39)^b	0.06 (8.75)^b	0.08(11.49)^b
	Organo-mineral associations	0.02 (2.51)^c	0.02 (5.63)^c	0.02 (6.07)^c

Note: The decreased amount of C (%) proportion to initial C of the aggregate fractions shows within the parenthesis. Different letters denote significant difference at $p < 0.001$.

5.3.6. Temperature effect on SOC mineralization

Soil C mineralization increased with increasing temperature in all the treatments (**Table 5.2**). However, a high MBC observed in temperature at 5 and 25 ^{0}C than 45 ^{0}C. This indicating that at 45 ^{0}C SOC decomposition is not coupled with the microbial population. This may be due to; firstly, some studies have hypothesized that temperature may physically alter the substrate making it more easily decomposable (Dalias et al., 2003; Rasmussen et al., 2006; Zogg et al., 1997), or the change in temperature may

result in shifts in the microbial community composition, resulting in microbes with the ability to metabolize substrates that were not used by members of community existing at lower incubation temperature (Schimel and Mikan, 2005; Zogg et al., 1997). In high temperatures (above the optimum for growth of the soil microbial community) allow to compare the decomposition ability of temperature dependence of microbial communities who living in different thermal environments. Alternatively, the increase of SOC decomposition may be due to the abiotic oxidation of SOC including the volatilization of organic compounds as described by Zimmermann et al. (2012a). More recently, Wagai et al. (2013) reported that the temperature sensitivity of organic C mineralization increased with increasing aromaticity of the light, mineral-free, organic C fractions in soil. This is supported with the LF-POM distribution in different land use systems discussed in section **5.3.1**. Forest and pasture soils observed higher amount of LF-POM than vineyards soils. Also among all the treatments, TOC depletion was least in vineyards soils due to the low labile SOC with compared to forest and pasture soils. Further, TOC lost was more significant at temperature level between 25-45 ^{0}C in forest and pasture soils. Therefore, these data confirms forest and pasture soils have decreased more C as these soils contained more labile fractions.

The TOC % mineralized of initial SOC during the incubation period at two temperature ranges are shown in the **Table 5.5.** The amount of C depletion is greater in the disaggregated soils compared to the aggregated soils in both 5-25 ^{0}C and 25-45 ^{0}C temperature ranges. This difference is almost twice in disaggregated soils than the aggregated soils at temperature range 5-25 ^{0}C in all the land use systems. Disaggregated soils have lost more C when increasing temperature from 5 ^{0}C to 25^{0}C. Therefore, decomposition of physically protected SOC mostly affected temperature range at 5-25 ^{0}C. The pasture soil has shown a significant difference of decomposition of between aggregated and disaggregated soils in both temperature ranges. This may be coupled with the disaggregation treatment has decreased more macroaggregate fraction in pasture soils than the forest and vineyard soils during the grinding process (**Figure 5.7**)

and this fraction greatly affected to TOC content of the bulk soil as discussed in the **section 5.3.3.**

Table 5.5. Mineralized TOC (%) of incubated soil at two temperature ranges.

Land use	**Treatment**	**Temperature**	
		5-25 ^{0}C	**25-45 ^{0}C**
Vineyards	Aggregated	3.38	3.12
	Disaggregated	5.93	4.87
Pasture	Aggregated	3.41	4.80
	Disaggregated	6.80	7.57
Forest	Aggregated	2.45	4.65
	Disaggregated	5.15	4.92

Several studies have described the effect of substrate availability on temperature responses of soil respiration (Bengtson and Bengtsson, 2007; Gu et al., 2004; Gershenson et al., 2009). These studies observed that decomposition rates increased with temperature when substrate availability and enzyme activity do not constrain reaction rates (Burke et al., 2003). Fissore et al. (2013) and Gershenson et al. (2009) reported glucose application has positive effect on temperature sensitivity of SOC and the sensitivity was significant in forest soils. Our results also comparable with these studies which observed the highest temperature sensitivity in forest soils among all land use systems (**Table 5.6**).

The temperature sensitivity on SOC decomposition within soil aggregates have not been studied broadly. Plante et al. (2009) reported that the physical protection of SOC is not sufficient to attenuate the temperature sensitivity of decompositions by using aggregates and crushed aggregates. They have reasoned the physical disruption treatments used in

experiments may have been insufficient to expose large amounts of physically protected SOC for decomposition. However, this study, observed smaller Q_{10} value for aggregated soils compared to disaggregated soils, which confirms that temperature sensitivity of SOC increased with increasing the compartmentalization of soil aggregates. However, the Q_{10} values of the disaggregated and aggregated soils were not significantly different ($p = 0.93$). Because, grinding treatment may not strong enough to disrupt all the aggregates to observe significant difference of Q_{10} values of C associated with aggregated and disaggregated soils.

Several studies observed of higher temperature sensitivity stable SOC pools than labile SOC pools (Biasi et al., 2005; Boddy et al., 2008; Bol et al., 2003; Conant et al., 2008a, b; Fierer et al., 2005; Ghee et al., 2013; Hakkenberg et al., 2008; Hartley and Ineson, 2008; Haddix et al., 2011; Larionova et al., 2007; Leifeld and Fuhrer, 2005; Rey et al., 2008; Vanhala et al., 2007). We also observed larger Q_{10} value for the HUM fraction than the LF-POM. The reason is SOC with complex molecular (e.g. recalcitrant SOC, adsorbed SOC and complexed SOC) characterized by low decomposition rates, high activation energies and, therefore, an 'inherently' high temperature sensitivity (von Lützow and Kögel-Knabner, 2009). Thus, organo-mineral associations and microaggregate observed larger Q_{10} values than macroaggregates. As we discussed in previous sections macroaggregates are stabilized with more labile SOC and the macroaggregae and organo-mineral associated SOC known to be more stable or recalcitrant form of SOM. We observed significant difference of the Q_{10} values among the aggregate frictions ($p = 0.02$) and the C fractions ($p = 0.003$).

Table 5.6. Averaged temperature sensitivity (Q_{10}) of TOC of bulk soil, macroaggregates (MA), microaggregates (MI), organo-mineral associations (OMA), LF-POM and HUM over the 180-days incubation period for temperature ranges 5-45 ^{0}C.

Land use	Treatment	Bulk soil	MA	MI	OMA	LF-POM	HUM
Vineyards	Aggregated	1.16	1.47	1.44	1.62	1.27	*
	Disaggregated	1.17				1.10	*
Pasture	Aggregated	1.20	1.16	1.37	1.69	1.08	1.44
	Disaggregated	1.29				1.01	1.41
Forest	Aggregated	1.27	1.25	1.46	1.55	1.02	1.52
	Disaggregated	1.35				0.95	1.21

* The over predicted values obtained from the Cubsit/MIR prediction models.

5.3.7. Spectral analysis

Few studies have used the abundance of the functional groups to predict the changes of the C fractions of soil used in incubation experiments (Calderon et al., 2011; Peltre et al., 2014). As we concluded from **Chapter 3**, the ratio of functional groups is important parameter to quantify the C fractions from the spectroscopy techniques. And this approach is used in this study to ascertain the changes of C fractions after the incubation period. **Figure 5.8.** shows the absorption differences obtained from the MIR spectra by subtracting the spectra taken 180-days after incubation from initial absorption spectra of vineyard, pasture and forest. The negative peaks show the decrease of specific functional groups after the incubation period. The large peak at 9500 nm and small peaks at 12438 nm and 10896 cm^{-1} have significantly decreased more than likely due to the decrease of soil carbohydrates and polysaccharides. The Carbohydrates are mixture of

polysaccharides and represents a significant pool in SOC, some 5-20% of the total SOC (Gregorich et al., 1994). Calderon et al. (2011) also observed the decreased of carbohydrates bands after 800-days incubated soils. Thus, carbohydrate is a major component of the light fraction and sensitive indicator of SOM decomposition (Gregorich et al., 1994). Also there are two sharp negative peaks at 6226 cm^{-1} and 2848 nm and these peaks are related to OH/NH and amide bands. Calderon et al. (2011) observed these peaks were prominent in light fractions' absorption spectra. The light fraction is closely related to plant residues and associated microbial decomposition products. Thus, OH/NH bands could be expected in this fraction (Haile-Mariam et al., 2008). Also, LF absorbance has affected by amide linkages could be due to absorbance of lignin-like materials of plant residues (Calderon et al., 2011). The spectral changes are greatly supported with the changes of the TOC of the incubated soils. The TOC % was highly reduced in forest and pasture soils and the negative peaks appeared to be prominent in forest and pasture soils. As discussed in the section **5.3.5**, macoaggregate C greatly influenced to the decreased of the TOC from the soils than the microaggregate C and organo-mineral associated-C. The functional groups related to the negative peaks are mostly due to the presence of carbohydrates and light fraction SOC. This confirms these negative peaks have been resulted mostly due to the decrease of the macroaggreagte C from the soils.

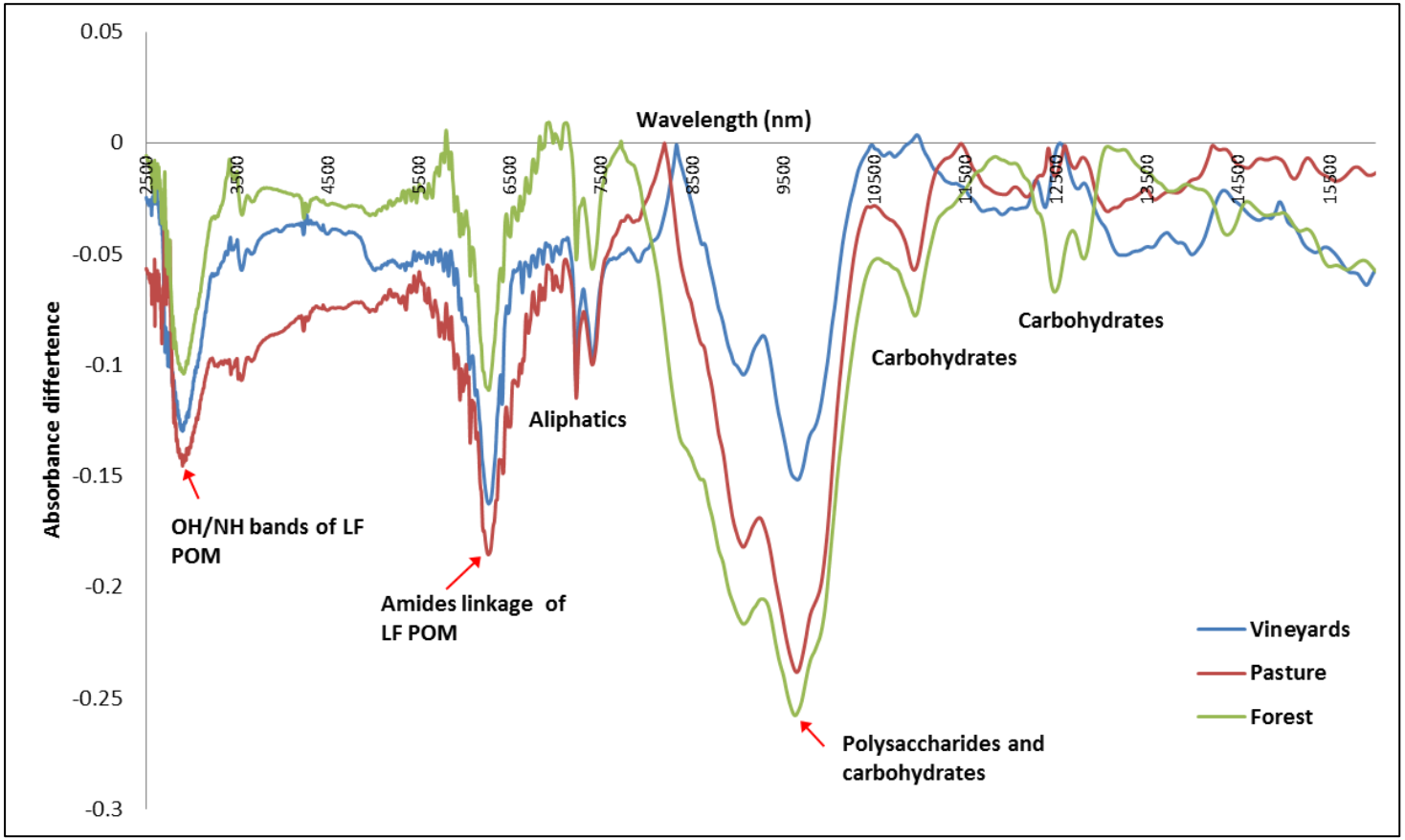


Figure 5.9. Absorption differences of the MIR spectra of vineyard pasture and forest soils after 180 days incubation.

5.3.8. The prediction of the carbon pools by MIR/Cubist model.

5.3.8.1. Accuracy of the predicted SOC fractions

In this study we assumed that RSOC (IOM) does not change after 180-days incubation. In Roth-C model the IOM pool is resistant to decomposition and having mean turnover rate up to 10,000 years (Coleman and Jenkinson, 1999). **Figure 5.9** illustrates the relationship between measured TOC % of soil and sum of the C fractions predicted 180-days after incubation. Theoretically, the sum of the predicted C fractions should be equal to measured TOC of the soils. A similar approach was used by Karunaratne et al. (2014) to assess MIR prediction capabilities of measurable fractions related to Roth-C model. The scatter plot indicates a good relationship between sum of the HUM, LF-POM and RSOC and measured TOC with 0.97 of R^2 together with all the land use systems (**Figure 5.9 (a)**). **Figure 5.9(b), (c), (d)** shows this relationship separately in each land use system. The predicted C fractions of the pasture soils were more accurate than vineyard and forest soils base on the R^2 and RMSE values. However, this study did not predict the DOC fraction as this fraction could not obtain accurate prediction model (see **Chapter 4)**. The initial DOC % of the soils is very low and we could assume this can be utilized by microbes very efficiently as this the most active C portion of the soils (Herbert and Bertsch, 1995). However, some studies has shown DOC content was increased after the incubation in pasture soil and decreased in cropland soils (Parfitt and Salt, 2001). Thus, the absence of the DOC fraction could be having a minor effect to the sum of the fractions when it correlating with the bulk TOC of the incubated soils. The amount of C of predicted HUM and LF-POM fractions were decreased with increasing temperature (**Table 5.7**). Also the C content of the fractions was low in disaggregated treatments. This indicates the treatment effect has well explained by the spectra when it predicting from the developed models in **Chapter 4**. The predicted values of LF-POM could be proved as reasonable values when considering the qualitative changes of the spectra described in the **section 5.3.7**. However, predicted HUM fraction showed few over predicted values in some treatments of vineyards soils (**Table 5.7)**. This fraction

was predicted using the S+C and S+A models which were obtained in the Chapter 3. These models showed some prediction errors as S+C with high RMSE and S+A with low RPIQ. Therefore, some prediction errors could be able to observe when these models using with an independent data set. However, this study could obtain reliable prediction results for the carbon fractions.. This indicates the MIR/Cubist models derived to predict the SOC fractions in the **Chapter 4** are a reasonable approach in Australian soils.

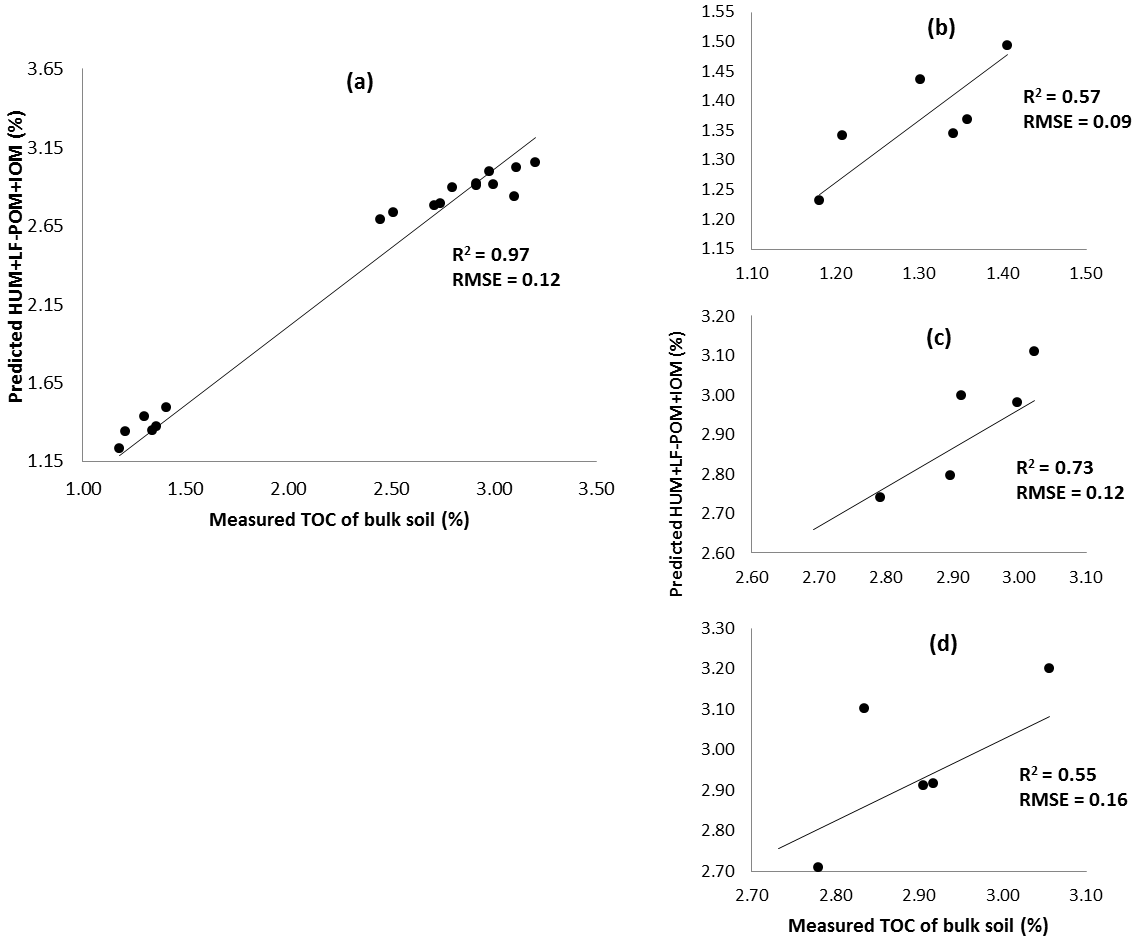


Figure 5.10. Relationships between measured TOC of bulk soil and sum of predicted HUM, LF-POM and IOM fractions in all the land use systems (a), vineyard (b), pasture (c) and forest (d).

5.3.8.2. Changes of the carbon pools during the incubation period

The predicted SOC fractions are shown in **Table 5.7.** LF-POM significantly decreased (average 79%) in all the treatments after 180-days incubation. Haile-Mariam et al., (2008) observed 65 % carbon lost from this fraction after 800-days incubation period. In this study the loss would be prime the microbial activities by the adding glucose to the systems. The labile pool has shown high temperature sensitivity when soil amended with glucose (Fissore et al., 2013; Gershenson et al., 2009). There was no significant difference in the loss of LF-POM among the land use systems. However, the loss was increased with increasing temperature. Also high amount of LF-POM has decreased in disaggregated soils than the aggregated soils. The C content of HUM fraction has depleted on average 7% from the initial. This decrease is considerably low compare with depletion of the C from the LF-POM. The HUM fraction is clay+silt and aggregate associated C and these fractions are physically and chemically protected by the microbial degradations. Thus, soil microorganisms are not capable of directly metabolizing structurally complexed recalcitrant substrates without help from extracellular enzymes that carry out the critical step of depolymerization (Gershenson et al., 2009). However, C content of the HUM fraction also slightly decreased when increasing the temperature and disaggregation. Wiesmeier et al. (2013) observed LF-POM and HUM fractions strongly controlled by temperature and precipitation. They observed a strong positive relationship with precipitation and a negative effect of temperature. In the present study we found that temperature greatly effecting to these fractions in soils of different land use systems.

Table 5.7. Mean prediction of SOC fractions by using 180-days incubated soil spectra.

Land use	Initial (%)			Treatment	Predicted after 180-days incubation (%)	
	HUM	POM	IOM		HUM	POM
Vineyard	1.17	0.26	0.14	Aggregated - 5 ^{0}C	1.25	0.11
				Disaggregated - 5 ^{0}C	1.14	0.08
				Aggregated - 25 ^{0}C	1.12	0.08
				Disaggregated - 25 ^{0}C	1.22	0.07
				Aggregated - 45 ^{0}C	1.19	0.01
				Disaggregated - 45 ^{0}C	1.04	0.05
Pasture	2.52	1.01	0.42	Aggregated - 5 ^{0}C	2.41	0.19
				Disaggregated - 5 ^{0}C	2.33	0.16
				Aggregated - 25 ^{0}C	2.40	0.18
				Disaggregated - 25 ^{0}C	2.22	0.15
				Aggregated - 45 ^{0}C	2.30	0.18
				Disaggregated - 45 ^{0}C	2.13	0.14
Forest	2.56	0.99	0.41	Aggregated - 5 0C	2.41	0.23
				Disaggregated - 5 ^{0}C	2.30	0.20
				Aggregated - 25 ^{0}C	2.22	0.20
				Disaggregated - 25 ^{0}C	2.26	0.24
				Aggregated - 45 ^{0}C	2.22	0.14
				Disaggregated - 45 ^{0}C	2.18	0.15

5.4. Conclusions

The distribution of the C associated with SOC fractions and aggregate fractions differed with land-use systems, where macroaggregate-C is larger in pasture and forest soils whereas the microaggregated and organo-mineral associated-C are larger in vineyard soils. Pasture and forest soils are enriched by more labile fractions and croplands by stable fractions. The turnover rate of the bulk soil TOC is affected by the land use systems. Forest and pasture soils showed largest carbon depletion compared to vineyard

soils. Soil aggregation has a great effect to protect SOC from microbial degradation. Soil aggregation has a great potential to protect SOC from the thermal kinetics reactions. Macroaggegate-C was shown to be more sensitive to temperature changes and microbial decomposition compared to the microaggrate-C and organo-mineral associated- C. The C % of LF-POM fraction is greatly utilized by the microbes and the MIR spectra are able to detect the change of the physically and chemical SOC fractions. The incubation studies are costly and time consuming to perform in both laboratory and field levels and the MIR models has shown great potential to be used to predict the SOC fractions, and therefore, this approach can be used to evaluate SOC turnover to populate the parameters of SOC models in future.

5.5. References

Bengtson, P., Bengtsson, G., 2007. Rapid turnover of DOC in temperate forests accounts for increased CO_2 production at elevated temperatures. Ecology Letter. 10, 783-790.

Biasi, C., Rusalimova, O., Mayer, H., Kaiser, C., Wanek, W., Barsukov, P., Junger, H., Richter, A., 2005. Temperature-dependent shift from labile to recalcitrant carbon sources of artic heterotrophs. Rapid Communication of Mass Spectrometry. 19, 1401-1408.

Boddy, E., Roberts, P., Hill, P.W., Farrar, J., Jones, D.L., 2008. Turnover of low molecular weight dissolved organic C (DOC) and microbial C exhibit different temperature sensitivities in Arctic tundra soils. Soil Biology and Biochemistry. 40, 1557-1566.

Bol, R., Bolger, T., Cully, R., Little, D., 2003. Recalcitrant soil organic materials mineralize more efficiently at higher temperatures. Journal of Plant Nutritional and Soil Science. 166, 300-307.

Bongiovanni, M.D., Lobartini, J.C., 2006. Particulate organic matter, carbohydrate, humic acid contents in soil macro- and microaggregates as affected by cultivation. Geoderma. 136, 660-665.

Bradford, M.A., Davies, C.A., Frey, S.D., 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters. 11, 1316-1327.

Burke, I.C., Kaye, J.P., Bird, S. P., Hall, S.A., Mcculley, R.L., Sommerville, G.L., 2003. Evaluating and testing models of terrestrial biogeochemistry: the role of temperature in controlling decomposition. In: Canham, C.D., Cole, J.J., Lauenroth, W.K., (Eds.), Models in Ecosystem Science, Princeton University Press, Princeton, pp254 -271.

Calderón, F.C., Reeves III, F.J., Collins, H.P., Paul, E.A., 2011. Chemical differences in soil organic matter fractions determined by diffuse-reflectance mid-infrared spectroscopy. Soil Science Society of American Journal. 75, 568-579.

Cambardella, C.A., Elliot, E.T., 1992. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Science Society of America Journal. 56, 777-783.

Cambardella, C.A., Elliott, E.T., 1994. Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Science Society of America Journal. 58, 123-130.

Chenu, C., Hassink, J., Bloem, J., 2001. Short-term changes in the spatial distribution of microorganisms in soil aggregates as affected by glucose addition. Biology and Fertility of Soils. 34, 349-356.

Coleman, K., Jenkinson, D.S., 1996. RothC-26.3-a model for the turnover of carbon in soil. In: Powlson, D.S., Smith, P., Smith, J.U., (Eds.), Evaluation of Soil Organic Matter Models, NATO ASI Series, Springer, Berlin. pp237-246.

Conant, R.T., Drijber, R.A., Haddix, M.L., Parton, W.J., Paul, E.A., Plante, A.F., Six, J., Steinweg, J.M., 2008a. Sensitivity of organic matter decomposition to warming varies with its quality. Global Change Biology. 14, 868-877.

Conant, R.T., Steinweg, J.M., Haddix, M.L., Paul, E.A., Plante, A.F., Six, J., 2008b. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology. 89, 2384-2391.

Creamer, C.A., Filley, T.R., Boutton, T.W. 2013. Long-term incubations of size and density separated soil fractions to inform soil organic carbon decay dynamics, Soil Biology and Biochemistry. 57, 496-503.

Elliott, E.T.,1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils1. Soil Science Society of America Journal. 50, 627-633.

Emerson, W.W., 1959. Stability of Soil Crumbs. Nature. 183, 538-538.

Fang, C., Moncrieff, J.B., 2001. The dependence of soil CO_2 efflux on temperature. Soil Biology and Biochemistry. 33, 155-165.

Fissore, C., Giardina, C.P., Kolka, R.K., 2013. Reduced substrate supply limits the temperature response of soil organic carbon decomposition. Soil Biology and Biochemistry. 67, 306-311.

Gee, G.W., Bauder. J.W., 1986. Particle-size Analysis. Methods of soil analysis, Part1, Physical and mineralogical methods. Second Edition, Agronomy Monograph, American Society of Agronomy, Madison, WI. pp383 - 411.

Ghee, C., Neilson, R., Hallett, P.D., Robinson, D., Paterson, E., 2013. Priming of soil organic matter mineralisation is intrinsically insensitive to temperature. Soil Biology and Biochemistry. 66, 20-28.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Soil structure and carbon cycling. Australian Journal of Soil Research. 32, 1043-1068.

Gregorich, E.G., Carter, M.R., Angers, D.A., Monreal, C. M., Ellert, B.H., 1994. Towards a minimum data set to assess soil organic matter quality in agricultural soils. Canadian Journal of Soil Science. 74, 367-385.

Gershenson, A., Bader, N., Chen, W., 2009. Effects of substrate availability on the temperature sensitivity of soil organic matter decomposition. Global Change Biology. 15, 176-183.

Gu, L., Post, W.M., King, A.W., 2004. Fast labile carbon turnover obscures sensitivity of heterotrophic respiration from soil to temperature: A model analysis. Global Biogeochemical. 18, 2003-2119.

Haddix, M.L., Plante, A.F., Conant, R.T., Six, J., Steinweg, J.M., Magrini-Bair, K., Drijber, R.A., Morris, S.J., Paul, E.A., 2011. The role of soil characteristics on temperature sensitivity of soil organic matter. Soil Science Society of America Journal. 75, 56-68.

Haile-Mariam, S., Collins, H.P., Wright, S., Paul, E.A., 2008. Fractionation and long-term laboratory incubation to measure soil organic matter dynamics. Soil Science Society of America. 72, 370-378.

Hartley, I.P., Ineson, P., 2008. Substrate quality and the temperature sensitivity of soil organic matter decomposition. Soil Biology and Biochemistry. 40, 1567-1574.

Herbert, B.E., Bertsch, P.M., 1995. Characterization of dissolved and colloidal organic matter in solutions: A review. In: McFee, W.W., Kelly, J.M., (Eds.), Carbon Forms and Functions in Forest Soils, Soil Science Society of America, Madison, WI, pp62-88.

Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soil: Measurement and turnover. In: Paul, E. A., Ladd, J.N., (Eds.), Soil Biology and Biochemistry. Marcel Dekker, New York, Basel, pp415-471.

Johna, B., Yamashitac, T., Ludwigb, B., Flessaa, H., 2005. Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma. 128, 63-79.

Kanazawa, S., Filip, Z., 1986. Distribution of microorganisms, total biomass, and enzyme activities in different particles of brown soil. Microbial Ecology. 12, 205 -215.

Kleber, M., Mikutta, R., Torn, M.S., Jahn, R., 2005. Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. European Journal of Soil Science. 56, 717-725.

Kirschbaum, M.U.F., 2006. The temperature dependence of soil organic matter decomposition-still a topic of debate. Soil Biology and Biochemistry. 38, 2510-2518.

Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., Leinweber, P., 2008. Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. Journal of Plant Nutrition and Soil Science. 171, 61-82.

Larionova, A.A., Yevdokimov, I.V., Bykhovets, S.S., 2007. Temperature response of soil respiration is dependent on concentration of readily decomposable C. Biogeosciences. 4, 1073-1081.

Lawrence, C.R., Neff, J.C., Schimel, J.P., 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biology and Biochemistry. 41, 923-1934.

Leifeld, J., Fuhrer, J., 2005. The temperature response of CO2 production from bulk soils and soil fractions is related to soil organic matter quality. Biogeochemistry. 75, 433-453.

Loughran, R.J., Elliott, G.L., Maliszewski, L.T., Campbell, B.L. 2000. Soil Loss and Viticulture at Pokolbin, New South Wales, IAHS Publications. 261, 141-152.

Mikutta, R., Kleber, M., Torn, M., Jahn, R., 2006. Stabilization of soil organic matter: Association with minerals or chemical recalcitrance? Biogeochemistry. 77, 25-56.

Mueller, C.W., Gutsch, M., Kothieringer, K., Leifeld, J., Rethemeyer, J., Brueggemann, N., Kögel-Knabner, I., 2014. Bioavailability and isotopic composition of CO_2 released from incubated soil organic matter fractions. Soil Biology and Biochemistry. 69, 168-178.

Nguyen, C., Henry, F., 2002. A carbon-14-glucose assay to compare microbial activity between rhizosphere samples. Biology and Fertility of Soils. 35, 270-276.

Olk, D.C., Gregorich, E.G., 2006. Overview of the symposium proceedings, 'Meaningful pools in determining soil carbon and nitrogen dynamics'. Soil Science Society of America Journal. 70, 967-974.

Parfitt, R.L., Salt, G.J., 2001. Carbon and nitrogen mineralization in sand, silt and clay fractions of soils under maize and pasture. Australian Journal of Soil Research. 39, 361-371.

Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry, Second (Eds.), Academic Press, SanDiego, pp273.

Pietikåinen, J. Pettersson, M., Bååth, E., 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. Microbiology Ecology. 52, 49-58.

Plante, A.F., Kougentakis, A., Sen, A.M., Slominski, C., Six, J., Paul, E.A., Conant, R. T., 2009. Does physical protection of soil organic matter attenuate temperature sensitivity ?. Soil Science Society of America Journal. 73, 1168-1172.

Poeplau, C., Don, A., 2013. Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma.192, 189-201.

Rasmussen, C., Southard, R.J., Horwath, W.R., 2006. Mineral control of organic carbon mineralization in a range of temperate conifer forest soils. Global Change Biology. 12, 834-847.

Saggar, S., Parshotam, A., Hedley, C., Salt, G., 1999. ^{14}C labelled glucose turnover in New Zealand soils. Soil Biology and Biochemistry. 31, 2025-2037.

Schimel, J.P., Mikan, C., 2005. Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. Soil Biology and Biochemistry. 37, 1411-1418.

Schneckenberger, K., Demin, D., Stahr, K., Kuzyakov, Y., 2008. Microbial utilization and mineralization of [^{14}C] glucose added in six orders of concentration to soil. Soil Biology and Biochemistry. 40, 1981-1988.

Segoli, M., De Gryze, S., Dou, F., Lee, L., Post, W.M., Denef, K., Six, J., 2013. AggModel: a soil organic matter model with measurable pools for use in incubation studies. Ecological Modeling. 263, 1-9.

Karunaratne, S.B., Bishop, T.F.A., Baldock, J.A., Odeh, I.O.A., 2014. Catchment scale mapping of measureable soil organic carbon fractions. Geoderma. 219-220.

Sivakesava, S., Irudayaraj, J., Demiric, A., 2001. Monitoring bioprocess of ethanol production using FT-MIR and FT-Raman spectroscopy. Journal of Microbiology and Biotechnology. 26, 185-190.

Sharabi, N.E., Bartha, R., 1993. Testing of some assumptions about biodegradability in soil as measured by carbon dioxide evolution. Applied and Environmental Microbiology. 59, 1201-1205.

Shrestha, B.M., Singh, B.R., Sitaula, B.K., Lal, R. Bajracharya, R.M., 2007. Soil aggregate and particle associated organic carbon under different land uses in Nepal. Soil Science Society of America. 71, 1194-1203.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil. 241, 155-176.

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry. 32, 2099-2103.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science. 33, 141-163.

Torn, M.S., Trumbore, S. E., Chadwick, O.A., Vitousek, P.M., Hendricks, D.M., 1997. Mineral control of soil organic carbon storage and turnover. Nature. 389, 170-173.

Vanhala, P., Karhu, K., Tuomi, M., Sonninen, E., Jungner, H., Fritze, H., Liski, J., 2007. Old soil carbon is more temperature sensitive than the young in an agricultural field. Soil Biology and Biochemistry. 39, 2967-2970.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry. 19, 703-707.

von Lützow, M., Kögel-Knabner, I., 2009. Temperature sensitivity of soil organic matter decomposition-what do we know?. Biology and Fertility of Soils. 46, 1-15.

Wagai, R., Kishimoto-Mo, A.W., Yonemura, S., Shirato, Y., Hiradate, S., Yagasaki, Y., 2013. Linking temperature sensitivity of soil organic matter decomposition to its

molecular structure, accessibility, and microbial physiology. Global Change Biology. 19, 1114-1125.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. American Journal of Alternative Agriculture. 18, 3-17.

White, R.E. 1969. On the measurement of soil pH. Journal of the Australian Institute of Agricultural Science. 35, 3-14.

Wiesmeiera, M., Schada, P., von Lützowa, M., Poeplaub, C., Spörleinc, P., Geuc, U., Hangenc, H., Reischlc, A., Schillingc, B., Kögel-Knabnera, I., 2014. Quantification of functional soil organic carbon pools for major soil units and land uses in southeast Germany (Bavaria) Agriculture, Ecosystems and Environment. 185, 208-220.

Yamashita, T., Flessa, H., John, B., Helfrich, M., Lud, B., 2006. Organic matter in density fractions of water-stable aggregates in silty soils: Effect of land use. Soil Biology and Biochemistry. 38, 222-3234.

Zimmermann, M., Leifeld, J., Conen, F., Bird, M.I., Meir, P., 2012. Can composition and physical protection of soil organic matter explain soil respiration temperature sensitivity? Biogeochemistry. 107, 423-436.

Zimmermann, M., Leifeld, J., Schmidt, M.W. I., Smith, P., Fuhrer, J., 2007. Measured soil organic matter fractions can be related to pools in the Roth-C model. European Journal of Soil Science. 58, 658-667.

Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., White, D.C., 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Science Society of America Journal. 61, 475-481.

Zsolnay, A., 1996. Dissolved humus in soil waters. In: Piccolo, A., (Eds.), Humic Substances in Terrestrial Ecosystems. Elsevier, Amsterdam. pp171-224.

Chapter 6: Discussion, conclusions and future work

6.1. Discussion

6.1.1. What have we learned about how soil aggregate relate to SOM?

The relationship between soil organic carbon and the presence of soil aggregates has long been studied (**section 2.3**) and this has led to the well accepted concepts of a soil aggregate hierarchy proposed by Tisdall and Oades (1982), and subsequently 'ideal' soil aggregate model represented by Jastrow and Miller (1998). Both the aggregate hierarchy model and ideal aggregate models state that different binding agents dominate soil aggregates within different size ranges and smaller organo-mineral associations are combined to form microaggregates, which are then housed in macroaggregates (**Figure 6.1a**). These expected differences in SOC within a hierarchy of aggregates has previously been determined by separating aggregate fractions and measuring the quantities of carbon they contain but comparatively less work has been undertaken to routinely determine the type, or functional groups, that characterise the carbon in these fractions (Jastrow and Miller, 1998; Six et al., 1998; Gale et al., 2000; Puget et al., 2000; Bongiovanni and Lobartini, 2006). Infrared spectroscopy is one technique that is employed to determine the different SOC functional groups and the analyse of aggregate fractions separated in this research showed that different functional groups did dominate macroaggregates, microaggregates and organo-mineral associations, respectively (**Figure 6.1b**).The SOC functional groups identified are compatible with the specific bonding mechanisms postulated in the different aggregate fractions of the aggregate hierarchy model. In particular, the ratios of the different functional groups in each of the aggregate fractions are diagnostic and in **section 3.3.4** were used to quantify the aggregate-associated carbon. The subsequent incubation experiment described in **section 5.3.7** used these newly established relationship and showed that the changes in these

ratios are a reliable attribute to quantify the SOC and aggregate turnover in the recently established Struc-C (2009) and AggModel (2013) carbon-aggregate turnover models.

Regarding the formation and stabilisation of soil aggregates much of research has focused on the effect of the labile (active) pool (POM, carbohydrates and MBC) to formation of soil aggregates (Bongiovanni and Lobartini, 2006; Gale et al., 2000; Jastrow and Miller, 1998; Puget et al., 2000; Six et al., 1998), and less attention has been given to the other fractions as described in **section 2.2,** intermediate pool (S+C and HF-POM) and inert pool (RSOC). Therefore, the need to determine the presence and association of the other SOC pools is demonstrated by the findings of Jastrow et al. (1996) where the average turnover time of C in free microaggregates was 412 years, whereas the average turnover time for macroaggregate-associated C was 140 years, while the turnover rate of the labile (active) pool ranges from months to a few years. This suggests that the turnover of SOC in aggregate fractions is not only controlled by the active pool but also by resistant and inert pools and to completely understand the SOC-aggregate relationship requires broader study (Besnard et al., 1996; Six et al., 1998; Yamashita et al., 2006).

To explore this SOC-aggregate relationship further this research quantified the S+C, HF-POM, LF-POM, RSOC and DOC of both bulk soil and aggregate fractions. These pools represent the labile, intermediate (S+C, and HF-POM) and inert pools (RSOC) conceptually described in soil carbon turnover models. Research in **Chapter 3** and **4** demonstrated that the proportion of each carbon pool contributes to the formation of macroaggregate, microaggregate and organo-mineral associations (**Figure 6.1c**). The S+C pool is the dominant pool among all the aggregate fractions, and organo-mineral associations form microaggregates which are then housed in macroaggregates as was to be expected **(Figure 6.1c**). The RSOC pool is mostly associated with microaggregates and organo-mineral associations. This fraction comprises more resistant material such as lignin, lipids, n-alkanes, and some charcoals (Zimmermann et al., 2007). Therefore, C associated with microaggregate and organo-mineral associations is more stable and has a long turnover time (Angers et al., 1997; Guggenberger et al., 1994; Kiem and Kögel-

Knabner, 2003) because C associated with these two aggregate fractions is more resistant to microbial degradation as observed in **Chapter 5**.

POM acts as binding material for soil aggregate fractions (Six et al., 2002) as it is mainly comprised of plant residues and microbial debris, such as fungal hyphae and spores (Besnard et al., 1996), and this SOC fraction needs to be maintained to ensure the renewal and longevity of soil aggregates. Recent concepts related to aggregate formation suggest that macroaggregates are formed with POM and over time the changes in SOC result in the formation and liberation of microaggregates (Six et al., 2000). As the SOC decays further the microaggregates in turn brake down liberating organo-mineral associations and RSOC. The macroaggregate fraction is dominated by LF-POM (34%) and this pool is relatively low in microaggregate (14%) fractions, which correlates with new ideas on the SCO-aggregate relationship, but the experimental approaches taken here cannot explicitly confirm this. Also, there is a strong positive correlation of POM and TOC of the macroaggregate fraction, and land use and increase in temperature correlated with the depletion of this LF-POM pool in macroaggregates. These observations are compatible with previous studies relating POM pools and aggregate dynamics described in **section 2.6.1.** This study observed that DOC does not significantly affect aggregate dynamics and stabilization as it is a very small proportion of the aggregate fractions of the soils sampled.

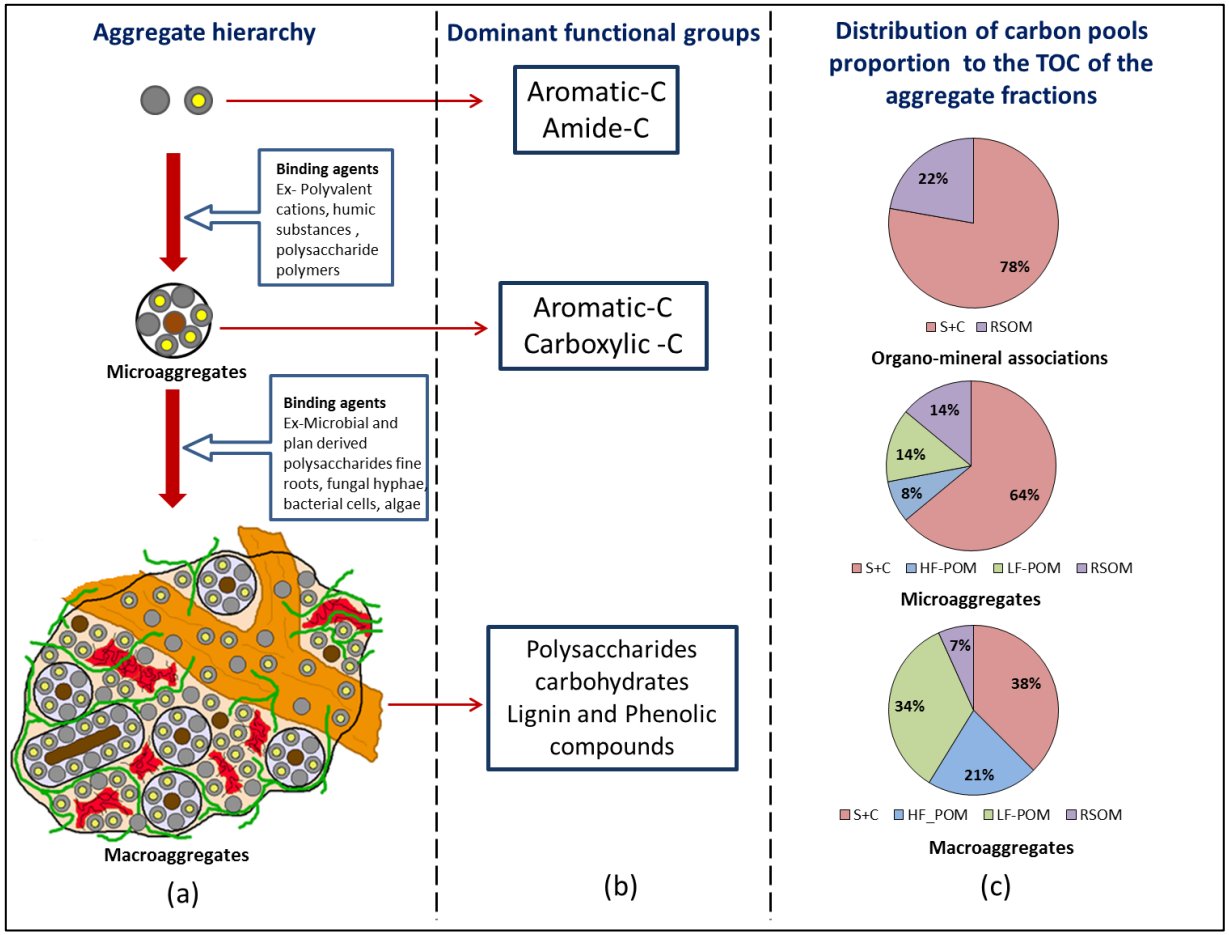


Figure 6.1. Schematic diagram of the traditional aggregate hierarchy (a) and the new research findings from the present study (b and c)

6.1.2. What does this study mean for soil carbon and aggregate turnover models?

This study measured both aggregate associated-C and SOC fractions as defined in soil carbon turnover models in both aggregate fractions and bulk soils (22 fractions). To my knowledge this is the first study that has fractionated both aggregate associated-C and carbon pools in detail (**Figure 6.2**).

With newly created models incorporating the dynamics of soil carbon turnover and the formation, interactions and decay of soil aggregates together it is becoming increasingly important to develop an efficient fractionation method to provided data that can be used in these models (Malamoud et al., 2009; Segoil et al., 2013) and this formed the focus of research in **Chapter 2**.

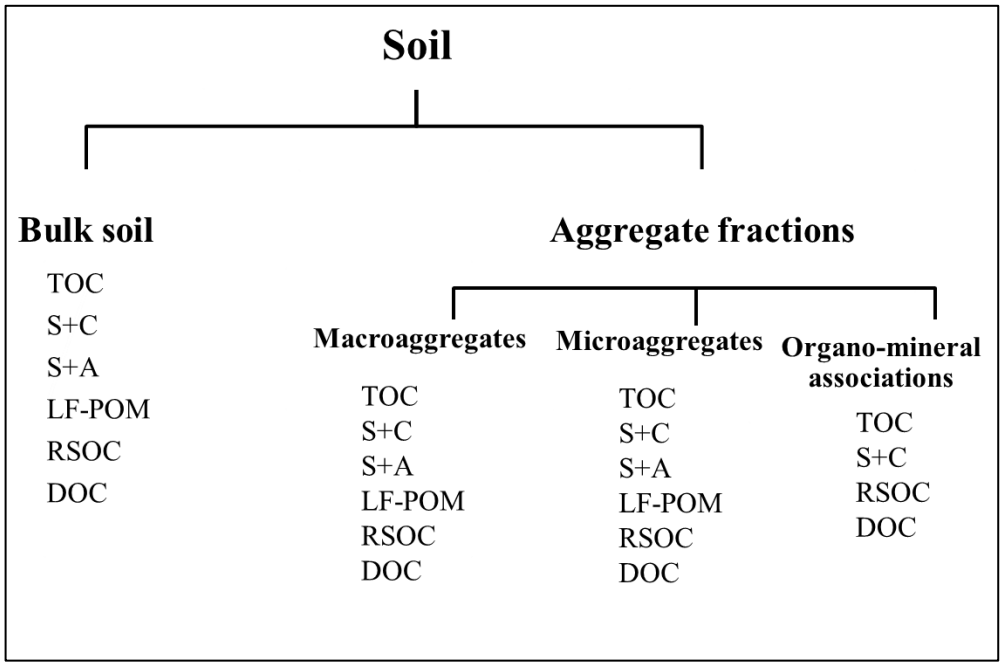


Figure 6.2. Physically separated aggregate and SOC fractions in Chapter 3 and 4.

The two models proposed to investigate the dynamics of both SOC and aggregations were Struc-C and AggModel. Currently, the Struc-C model is primarily used to simulate only the TOC and does not use any measures of carbon fractions. However, one of the disadvantages of this model was lack of measured C fractions to validate the model. This study separated Type1, 2 and 3 aggregate fractions (organo-mineral, microaggregate and macroaggregate) and measured C as defined AC1, AC2 and AC3 (**Table 6.1**). The AggModel (Segoli et al., 2013) used 10years of published data from 34 laboratorial incubations studies which were done to observe different parameters of and it was possible dynamics, these and the separated aggregate and carbon fractions in this study were related to the pools defined in the AggModel, being; aggregate associated-C (M, m, u) and aggregate associated carbon fractions (MAOM-M, MAOM-m,s MAOM-u, POM-m, POM-m) and it was possible to relate with our measured fractions. Further, in **Chapter 5** observed the turnover of the aggregate fractions and carbon pools in different land use systems under constant moisture level at different temperature levels. Thus, we have measured well-defined series of carbon fractions and quantified the turnover of them. These data will greatly support populating and validating of both aggregate and carbon turnover models in future. However, this study did not use measured data to populate any aggregate or carbon turnover models to observe the aggregate and C dynamics, as this thesis was more focused on increasing the efficiency of soil carbon measurements.

Table 6.1. Potential to use measured fractions of bulk soil (bulk), macroaggregate (Macro), microaggregates (Micro) and organo-mineral associations (Organo) for Struc-C, AggModel and Roth-C models.

Measured fraction	Struc-C	AggModel	Roth-C
Macroaggregate-C	Type 3 (AC3)	M	
Microaggregate-C	Type 2 (AC2)	m	
Organo-mineral association-C	Type 1 (AC1)	u	
Bulks soil-S+C			HUM
Bulk soil-S+A			
Bulk soil-LF-POM			DMP+RMP
Bulk soil-DOC	FOM (DMP+RMP)		
Bulk soil-RSOM			IOM
Macro-S+C		MAOM-M	
Macro-S+A		POM-M	
Macro-LF-POM			
Macro-RSOM			
Macro-DOC			
Micro-S+C		MAOM-m	
Micro-S+A		POM-m	
Micro-LF-POM			
Micro-RSOM			
Micro-DOC			
Oragno-S+C		MAOM-u	
Organo-RSOM			
Organo-DOC			

6.1.3. What does this study mean for quantification different SOC fractions?

One of the main objectives of this study was to increase the efficiency of soil carbon fraction measurements, i.e. remove the need to physically separate SOC fractions, and this involved investigating the ability of spectroscopic techniques to quantify carbon fractions as an alternative approach. In doing so, this study demonstrates that MIR is better suited than NIR for quantifying C in aggregates, however, the use of a larger number of samples in future research might to improve results with NIR.

It is known that conventional fractionation procedures used to separate both aggregates and carbon pools are time and cost consuming. **Figure 6.3.** shows a time comparison of using traditional fractionation procedures and MIR spectroscopy for 180 samples. Nine months were spent to separate the fractions using combined wet sieving and carbon pool fractionation methods as described in the **Chapter 3** and **4**. However, a week was needed for sample preparation, spectra collection, and spectra analysis by MIR. Because NIR has the immediate potential to be portable compared to it would be even more efficient for both field and laboratory conditions if we could the NIR predictions. This study observed about 90% of SOC pools listed in the **Table 6.1.** have a great potential to be quantified using MIR spectroscopy (**Chapter 3 and 4)**. This study was not able to obtain satisfying prediction results for RSOC and DOC pools as I identified some quantification problems with those pools. **Chapter 5** used the developed Cubist/MIR model to predict the turnover of the carbon pools of independent data set and obtained reliable prediction results from the models. This confirmed there is a great potential use of spectroscopic techniques to quantify the carbon pools in both aggregate and carbon turnover models. Therefore, this study will greatly help to reduce the costs and time spent to separate the carbon pools and use of carbon turnover model to predict the carbon dynamics in future.

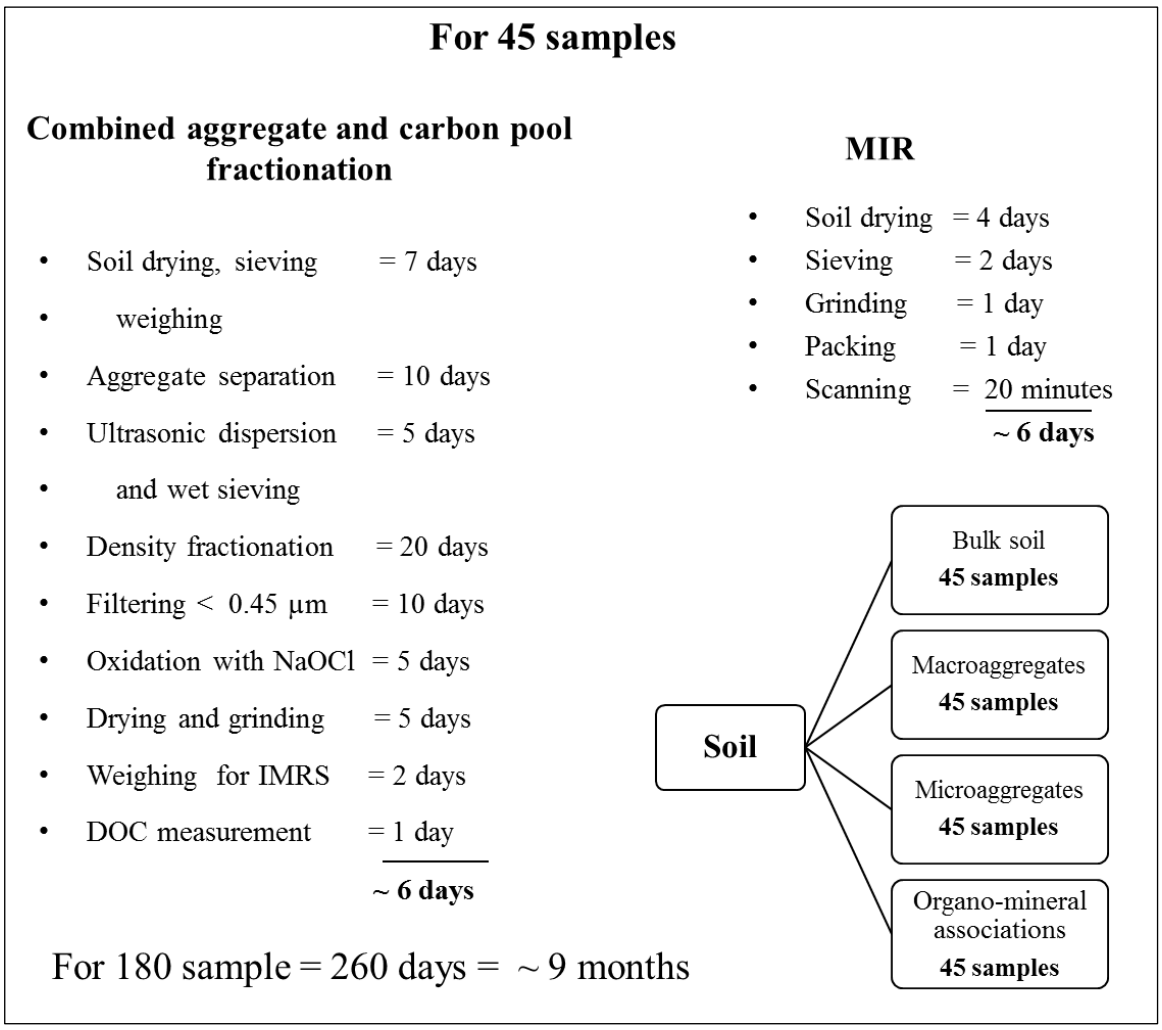


Figure 6.3. A comparison of time consumed for MIR measurement and fractionation procedures to separate aggregate and carbon pools for 45 samples.

6.2. Overall conclusions

The work described in this thesis focused on the development of an efficient method to quantify soil carbon pools using spectroscopic techniques. The overall outcomes of this study are the following:

- Using both mid-infrared (MIR) and near-infrared (NIR) spectroscopy is possible to quantify the aggregate associated carbon and considered specific functional groups that relate to aggregate size fractions.

- Polysaccharides, carbohydrates and aliphatic-C are the major functional groups associated with macroaggregates. Aromatic-C, carboxyl-C and aliphatic-C are significantly associated in microaggregates and organo-mineral associations. These specific functional groups dominated aggregate fractions as aggregates are stabilized within different carbon pools. The S+C pool dominates all aggregate fractions. The LF-POM is significantly associated with macroaggregates while RSOC is mostly associated in microaggregates and organo-mineral associations. There is a great potential to quantify aggregate associated-C pools using MIR spectroscopy.

- As expected findings of the incubation study supported that different management strategies affect the physical and chemical protection of the SOC, where the 'active' carbon pools are larger in grassland and forest soil and the resistant carbon pools are dominant cultivated soil Use of the spectral features are more efficient to identify the turnover of the carbon pools. The changes of the specific functional groups observed in the bulk spectra indicate changes in the pools.

- Temperature significantly effects physically protection of the carbon in aggregate fractions. The decrease of the physical protection of soil carbon is greatly influenced by the temperature and microbial decomposition. The depletion of the

C from soil mainly affects by the decrease of macroaggregate associated carbon from the soil. This decreased is resulting due to the loss of more LF-POM from the macroaggregates. Thus, the macroaggregate associated-C is more vulnerable to microbial decomposition than the other C pools. These carbon changes are directly observed by the changes of the specific functional groups from the MIR spectra. Thus, MIR spectra give both qualitative and quantification estimates of the soil C decomposition.

6.3. Future work

The notion that there is a relationship between soil organic carbon (SOC) and the presence of soil aggregates has long been established, and the proposition and aggregate hierarchy of Tisdall and Oades (1982) and the ideal aggregate of Jastrow and Miller (1998) infers that the type or quality of SOC is intrinsic to different aggregate fractions. Currently, SOC is discriminated in two ways using fractions that are used to populate soil carbon turnover models, or the soil carbon found within aggregate fractions that are isolated when studying aggregate stability. The research presented here has confirmed that spectroscopic techniques can be used to discriminate the functional groups (or quality) of the carbon and in doing so the resulting spectra can be used to quantify the present carbon. The new findings here have demonstrated the potential to predict the SOC without the physical fractionation, and as described in the literature review, has the potential to save time making the analysis of soil carbon fractions to become part of routine soil analysis. Future work to improve these predictions is the increase in the number of soil samples representing a range of different soil conditions and biophysical environments, all of which affects the SOC quality and its relationship with soil aggregates. This will contribute to the development of a global data set for predicting SOC fractions from bulk soil, much in the same way research is continuing to produce a global data set for the prediction of other soil properties using infrared spectroscopic techniques.

Currently, Struc-C (Malamoud et al., 2009) and AggModel (Segoil et al., 2013) have described two aggregate formation processes, where the basic concept of Struc-C is organo-mineral associations bound together to form microaggregates and clustering of microaggregates forms macroaggregates (**Figure 6.4**). Alternatively, AggModel adopts the concept described by Six at al. (2000), i.e. the breakdown of macroaggregates formed around SOC results in the liberation of microaggregates and these microaggregates either breakdown further or become the building blocks of the next cycle of the macroaggregate formation (**Figure 6.4**). Both aggregate formation processes are mediated by SOC and there is still debate on which and/or if both processes are responsible for the initiation of soil aggregates. To investigate these processes there are a few studies that have reported the use of isotope ^{13}C tracers (Angers et al., 2007; Jastrow et al., 1996) and have concluded that young or fresh SOC is enriched in macroaggregates and that carbon is found in microaggregates. This research indicates that macroaggregates form and then give rise to microaggregates. The research presented here discriminates the functional groups that associate the macroaggregates; being a higher portion of polysaccharides, carbohydrates and aliphatic-C, while aromatic-C and carboxylic-C proportionally dominates microaggregates and the organo-mineral associations. Yet, there are no studies that have reconciled the functional groups present and the assessment to carbon maturation overtime investigated using isotope ^{13}C tracers. This will require the assessment of the carbon functional groups in each fraction using the relative changes in absorption ratios between the bulk soil and aggregate fraction spectra. This will enable us to have an understanding of the development and longevity of macro- and microaggregates and explore more deeply the aggregate formation pathways illustrated in **Figure 6.4**. Subsequently, this can be used to improve the assumed relationships that have been developed to frame Struc-C and/or AggModel.

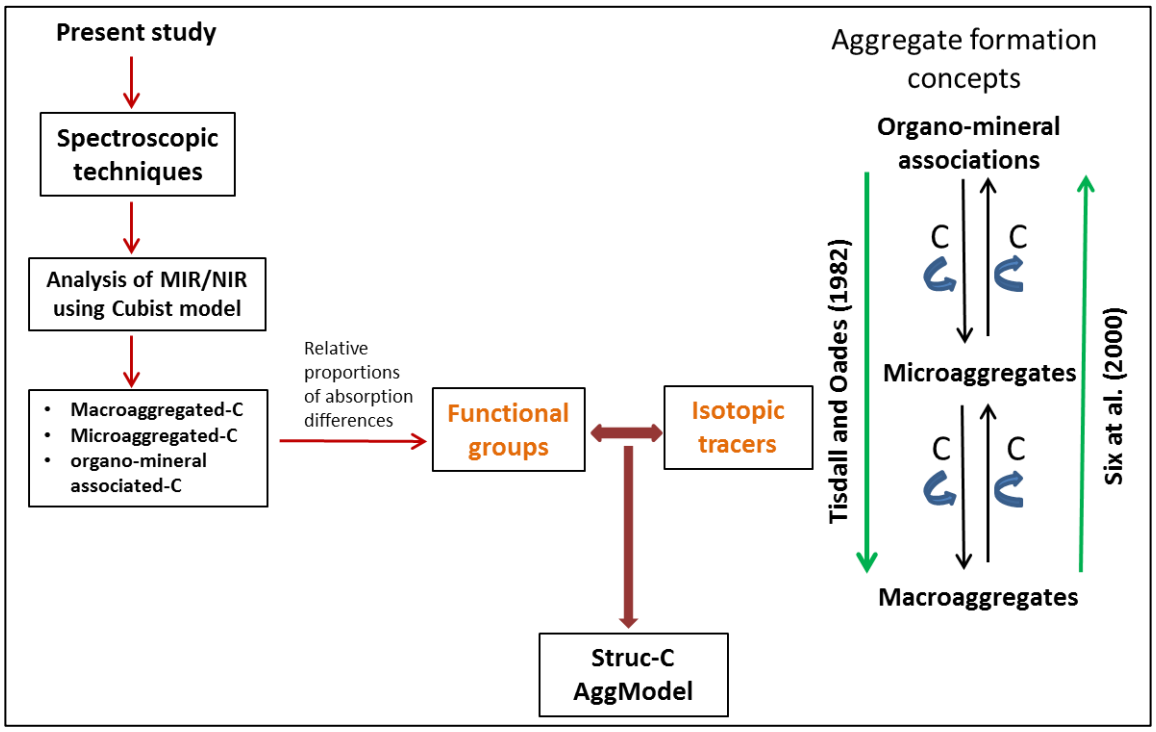


Figure 6.4. Focuses on future studies relate to the aggregate-C relationships

Finally, while both Stuc-C and AggModel can describe and quantify the process and changes in soil aggregation there is also a need to develop these models so their outputs can be used by land managers concerned with soil physical attributes resulting from carbon inputs (**Figure 6.3.1**). To do this future research will benefit from incorporating relevant data representing the different biophysical and management conditions of different cropping and pasture systems. These improved models will predict change in the soil structure through its aggregation which affects water holding, root depth and nutrient availability, and using spatial data the predicted changes in soil structure can be mapped across the field enabling a more precise approach to within field management.

6.4. References

Angers, D.A., Recous, S., Aita, C., 1997. Fate of C and nitrogen in water-stable aggregates during decomposition of $^{13}C^{15}N$ labeled wheat straw in situ. European Journal of Soil Science. 48, 295-300.

Besnard, E., Chenu, C., Balesdent, J., Puget, P., Arrouays, D., 1996. Fate of particulate organic matter in soil aggregates during cultivation. European Journal of Soil Science. 47, 495-503.

Bongiovanni, M.D., Lobartini, J.C., 2006. Particulate organic matter, carbohydrate, humic acid contents in soil macro- and microaggregates as affected by cultivation. Geoderma. 136, 660-665.

Gale, W.J., Cambardella, C.A., Bailey, T.B., 2000. Root-derived carbon and the formation and stabilization of aggregates. Soil Science Society of America Journal. 64, 201-207.

Guggenberger, G., Christensen, B.T., Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. European Journal of Soil Science. 45, 449-458.

Jastrow, J.D., Boutton, T.W., Miller, R.M. 1996. Carbon dynamics of aggregate associated organic matter estimated by Carbon-13 natural abundance. Soil Science Society of America Journal. 60, 801-897.

Jastrow, J.D., Miller, R.M., 1998. Soil aggregate stabilization and carbon sequestration: feedbacks through organo-mineral associations. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A., (Eds.), Soil Processes and the Carbon Cycle. CRC Press, Boca Raton. pp207-223.

Kiem, R., Kögel-Knabner, I., 2003. Contribution of lignin and polysaccharides to the refractory carbon pool in C-depleted arable soils. Soil Biology and Biochemistry. 35, 101-118.

Puget, P., Chenu, C., Balesdent, J., 2000. Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates. European Journal of Soil Science. 51, 595-605.

Segoli, M., De Gryze, S., Dou, F., Lee, L., Post, W.M., Denef, K., Six, J., 2013. AggModel: a soil organic matter model with measurable pools for use in incubation studies. Ecological Modelling. 263, 1-9.

Six, J., Conant, R. T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil. 241, 155-176.

Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal. 62, 1367-1377.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science. 33, 141-163.

Zimmermann, M., Leifeld, J., Abiven, S., Schmidt, M.W.I., Fuhrer, J., 2007. Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis. Geoderma. 139, 171-179.