

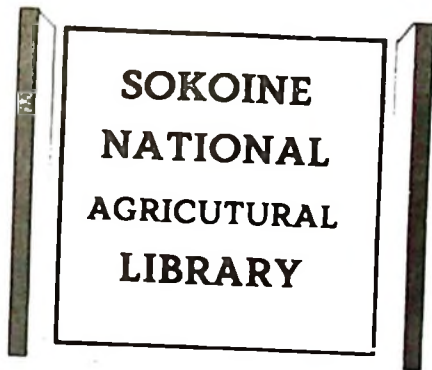


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Localization of mineralizable organic carbon using X-Ray CT



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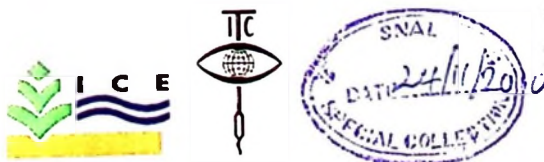
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Master dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in Physical Land Resources

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Gent,

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A large, stylized handwritten signature in black ink, consisting of several overlapping loops and a long horizontal stroke extending to the right.A smaller, more legible handwritten signature in black ink, appearing to read 'Sanga' followed by a stylized 'ga'.

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I wish to dedicate this thesis to my parents who sacrificed their life for me.

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Hilda Gerald Sanga

ABSTRACT

The sensitivity of organic C (OC) to decomposition depends not only upon its chemical composition but also on its location within the soil matrix. However, the precise location of OC mineralization is not clearly known. This Master thesis research's objective was to investigate the role of soil pore structure on the organic matter (OM) decomposability in a model sandy loam soil. To achieve this, the influence of different artificial operations (compaction, artificial change in texture, acidification, and OM application) on soil pore size distribution, decomposer group community (assessed by the fungal: bacteria ratio) and C mineralization was studied. Two major experiments were included. In the first one, manipulated soil samples were incubated in larger tubes for five weeks. A second incubation experiment was set up to follow C mineralization of added C-sources (grinded sawdust or grass particles) in artificially manipulated soil with differing pore size distribution and decomposer community. Soil samples were incubated in small tubes (1cm diameter*1cm height) for five weeks and the evolving CO₂ gas was analyzed by a Gas chromatography method. Samples were then scanned after incubation by X-ray computed tomography (CT) and following image processing, the total pore volume and % volume of different pore size classes were calculated. Correlation analysis was used to investigate the relation between the % pore volume of five pore classes (10-200, 210-400, 410-600, 610-800 and >800 μm equivalent sphere diameter) and the cumulative C mineralization after 5 weeks.

Artificial manipulation of the sandy loam soil, through compaction at BD 1.6 g cm^{-3} and artificial change in texture from coarse sand: fine sand: silt and clay (CS: FS: S&C) ratio of 10:40:50 to 15:50:35 and 20:60:20 was found to reduce the total soil porosity. Soil compaction at BD 1.6 g cm^{-3} reduced the proportion of macropores (pores with pore neck diameter 30-300 μm) while artificial change in soil texture found to affect mainly the distribution of micropores (pores with pore neck diameter 0.2-15 μm) and to a lesser extent the macropores. Soil amendment with grass material found to increase the total soil porosity. Artificial change in soil texture, soil acidification to pH 4.3 and additions of sawdust material compared to grass changed the microbial community towards more fungi oriented. This suggests these artificial soil operations to be usable for manipulation of soil pore structure and the microbial community.

In the second experiment, very pronounced interaction effects on C mineralization between substrate type and the artificial changes in soil pore structure were found. First an interaction

effect between soil compaction at $BD\ 1.6\ g\ cm^{-3}$ compared to $BD\ 1.3\ g\ cm^{-3}$ and substrate type was present as the reduction on C mineralization was more pronounced in grass amended soil than in sawdust amended soil. Similarly, a very pronounced interaction effect between artificial change in texture and substrate type was also noted as addition of sawdust strongly reduced the net substrate derived C mineralization, while grass addition did not. C mineralization from native SOM positively correlated to % pore volume of the 200-600 μm , class implying the dominance of mineralization in intermediate sized pores. On the other hand, a positive correlation between C mineralization and % volume of 610-800 μm pores with both grass and sawdust addition demonstrates the importance of larger pores for substrate decomposition. An inhibiting effect of soil compaction on C mineralization was observed from pF and X-ray CT data and this was likely related to the effect of macropore reduction and probably aeration. The negative influence on C mineralization of artificial change in soil texture and the associated reduction in the microporosity only with addition of sawdust but not with grass seems to be related to observed differences in microbial community involved in decomposition of both substrates. Interestingly but complex interactions between soil pore structure and substrate type were demonstrated in this thesis. Pronounced negative or positive correlations between individual pore size class volumes and C mineralization could be established and this indicates their role in the OM decomposition process.

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List of Abbreviations

SOM	Soil organic matter
SOC	Soil organic carbon
OM	Organic matter
OC	Organic carbon
CEC	Cation exchange capacity
PLFA	Phospholipids fatty acids
POM	Particulate organic matter
MBC	Microbial biomass carbon
CT	Computer tomography
SWRC	Soil water retention curve
WFPS	Water field pore space
kEC	Extraction efficient
VOI	Volume of interest
GC	Gas chromatography
CS:FS:S&C	Coarse sand: Fine sand: Silt and clay
RETC	Retention curve
BD	Bulk density
SD	Sawdust
GC	Grass

CHAPTER 1: INTRODUCTION

The storage and cycling of soil organic matter (SOM) is essential to the functioning of terrestrial ecosystems (McCarthy et al, 2008). SOM plays many important roles including formation and improvement of soil structure stability, increasing cation exchange capacity (CEC) of the soil, acting as a source of energy to micro organisms and thus enhancing microbial activities. It also serves as nutrients reserve in the soil, reduces surface crusting and compaction and thus enhancing water infiltration, percolation and water holding capacity of the soil. Soil Organic carbon (SOC) is stored when the rate of OC-input exceeds mineralization process (Blum et al, 2008). The balance between OC inputs and mineralization processes is controlled by biotic and abiotic factors such as soil pH, quality and types of organic matter, soil compaction, pore size distribution and soil structure. These factors influence the microbial distributions and environmental conditions favorable for microbial activities.

Soil structure is a key factor in the functioning of soil, through its ability to support plant and animal life, and moderate environmental quality with particular emphasis on soil carbon sequestration and water quality. Soil structure describes the arrangement of the solid parts of the soil and of the pore space located between them (Marshall and Holmes, 1979). Soil structure and its dynamics including the formation, stabilization, and destabilization of aggregates exert important controls on the turnover of C and N in soil (Ladd et al, 1993; McCarthy et al, 2008). This is because the pore space distribution and particularly the habitable pore spaces have a major impact on the distribution of bacteria, fungi and micro- and mesofauna (Juma, 1993).

The pore system can be viewed as the residence in which soil organisms are distributed as well as the environment in which interactions between organisms and their substrates are constrained (Strong et al, 2004). Protection of soil OM within OM–mineral assemblages is generally considered to result from the biotic exclusion (e.g. mineral encapsulation), wherein OM held among mineral grains excludes either organisms (Kilbertus, 1980; McCarthy et al, 2008) or their enzymes (Mayer 1994; McCarthy et al, 2008). Therefore the sensitivity of OC to decomposition depends not only upon its chemical composition but also its location within the soil structure. Soil structure provides OC with physical protection, to the extent which varies between structural units, and in return OM contributes to soil structural stability.

Elucidating the role of soil matrix in SOM decomposability is therefore important in order to quantify the amount of OC which is physically protected against mineralization. A key unknown is the precise location of OC mineralization within the soil matrix. More specifically, identification is needed of the role and occurrence of pore size classes that either promotes physical OC protection or in which decomposition occurs.

To date, most of the researches which have been done to study the role of soil structure in protecting the SOM have relied upon some form of fractionation technique to separate aggregates from the bulk soils (Wilson et al, 2008). This approach, however, has its own weakness since in the process most of the soil structures are distorted. Moreover, in this process the spatial relationships between aggregates are lost and the true spatial distribution of pores or C mineralization is obscured (Blum et al, 2008). Therefore, to study the impact of soil structure upon OC-storage alternative methods that minimize disturbance to the soil structure are needed, allowing studying organic matter decomposition within undisturbed soil structures. X-ray computer tomography provides exciting opportunities to study the dynamics of soil structure from undisturbed soils (Wilson et al, 2008).

This master thesis research aims to investigate the role of spatial heterogeneity of soil pore structure on the OM's decomposability. Analysis of relationships between the volumes of different pore size classes and various soil properties can provide an understanding of soil organization within the framework of the pore system (Strong et al, 2004). In this research, the methodology encompasses a correlation analysis between parameters describing pore size distribution (volumes of pore size classes) on one hand and C decomposition and microbial activities on the other. A first experiment will be set up to investigate how different artificial operations succeed in manipulating pore size distribution. Factors which regulate the distribution of OM and decomposing microorganisms in different pore size classes like soil compaction, pH, Particle size distributions and OM types will be studied. Soil microbial carbon and the Bacteria to Fungi ratio from Phospholipids Fatty Acid (PLFA) analysis will be investigated. Different pore size classes formed after incubation of the reconstructed sandy loam soil will be investigated from water retention curve and by physical fractionation.

A second primary incubation experiment builds on the first one and is designed to obtain different pore size class distributions for a model sandy loam soil. C mineralization of added C-sources (added as particulate organic matter POM) will be followed up in custom made small (1cm diameter *1cm height) metal soil cores. The volume % of different pore size

classes will be determined by X-ray computer tomography. The study will help to improve our understanding on physical protection of OC in small pores by investigating OC within thin section, thus advancing from the over-reliance of soil-fractionation techniques that destroy much of the soil structure.

CHAPTER 2: LITERATURE REVIEW

2.1 Interactions between soil structure and SOM

Soil Organic Matter (SOM) promotes stabilization of soil aggregate since during its decay it acts as a glue to hold soil particles together. Aggregation results from the rearrangement of particles, flocculation and cementation processes (Duiker et al, 2003). Aggregation is mediated by soil organic carbon (SOC), biota, ionic bridging, clay and carbonates. Adhesion between particles is via electrostatic force (flocculation) or cementing substances, such as organic matter and minerals. SOM thereby influences the pore size distribution and the distribution of the micro organisms. Ingham (2000) reported that soil microorganisms tend to occur wherever organic matter occurs. Changes in SOM content have an impact on soil porosity and pore size distribution (Kahle et al, 1992). Scheffer and Schachtschabel et al (1989) reported that in fine textured soil, reduction in organic matter content results in reduction of micropores while in course textured soil, reduction of organic matter content increases the micropores. Messing et al (1997) reported the formation of more macropores ($>75\mu\text{m}$) at higher Soil C levels in sandy soils. Kirchmann et al (1999) observed a non uniform increase of soil porosity with increasing SOC level, but they also observed no effect on water storage capacity of the soil.

On the other hand, soil structure influences SOM decomposition and mineralization through its effect on the soil pore system, thus determining the location and distribution of the SOM and microorganisms. Soil structure provides physical protection to SOM in three ways:

- i) Through adsorption of organics to surfaces of clays or coatings of organics by clay particles (Tisdall and Oades, 1982)
- ii) Through entrapment of organics in small pores in aggregates inaccessible to microbes (Elliott and Coleman, 1988)
- iii) Through its effect on grazing intensity on microbes

Elliott et al (1980) reported that predation of microbes by protozoa and nematodes are important mechanisms of nutrient turnover in soil. A large number of bacteria may occupy pores $< 3\mu\text{m}$ (Kilbertus, 1980) while large organisms like nematodes and protozoa are restricted in larger pores. This implies the physical separation of large part of bacteria population from protozoans and nematodes in the soil (Postma and Van Veen, 1990).

Heijnen et al (1988) reported a higher predation in sandy soil than in loam and clay soils, due to a large amount of larger pores in the sandy soil than in the loam and clay soils. This was related to the research by Hassink et al (1993) which indicated that the higher grazing pressure in sandy soils strongly affects the nitrogen (N) mineralization rate. Verberne et al (1990) reported a more rapid net mineralization and decomposition of plant materials in sandy soils than in clay soils. In his observation he noted that lower net mineralization in clay soil was partly caused by greater physical protection of SOM and microbial biomass. Soil structure can also affect the relative contribution of bacteria and fungi to the total microbial biomass. Fungi can habit pores with different sizes compared to those where bacteria can be found since the fungal hyphae can vary considerable in diameter, and they can bridge inhabitable spaces in soil to reach habitable pore space.

2.2 C Mineralization in the soil.

Soil C and N mineralization rates and soil microbial biomass carbon (MBC) are soil biological properties that determine the soil nutrient availability and SOM dynamics. Decomposition of organic matter is largely a biological process that occurs naturally and its speed is determined by three major factors: soil organisms, the physical environment such as soil pH, texture, pore size distribution and the quality of the organic matter (Brussaard, 1994). Changes in these factors will result in either positive or negative effect on C mineralization. The experimental set-up in this master thesis focuses on effect of pH, compaction, soil texture and organic matter (OM) quality on C mineralization. These first three determining factors will be discussed in 2.2.1, 2.2 2 and 2.2.3 sections below.

2.2.1 Soil compaction and organic matter decomposition

Soil compaction is one of the major problems facing modern mechanized Agriculture. Soil compaction occurs as a result of intensive tillage by heavy machines or by trafficking with construction machinery and also due to animal trampling in cultivated fields. Breland et al (1996) reported that, soil compaction reduces the total soil pore volume and changes the pore size distribution towards a higher percentage of small pores. In their study they observed that soil compaction reduces the volume of pores with neck diameter $> 30\mu\text{m}$, i.e. pores available to nematodes, from 30.4 to 14.6% of total bulk volume, and volume of pores $< 3\mu\text{m}$ i.e. pores that are unavailable to cellular organisms or available only to bacteria and fungi increased from 12.7 to 15.6%. It was suggested that in these small pores, organic materials may be

physically protected against microbial attack and microorganisms may be inaccessible to predating protozoa and nematodes.

Soil compaction may result into retardation of organic matter decomposition. De Neve et al (2000) reported that, increase in soil compaction starting from bulk density of 1.5 Mg m^{-3} affects some microbial driven processes such as nitrification and carbon mineralization. In their study using a loamy sand soil, they observed a strong decrease in C mineralization rate in a compacted soil with a bulk density of 1.6 Mg m^{-3} for amended soil. Reduction of C mineralization in compacted soil led to accumulation of organic matter and there was no effect of compaction on N mineralization observed and thereby the accumulated OM had a higher C: N ratio than OM accumulated in non compacted soil. Vander Linden et al (1989) observed a decrease in C and N mineralization from native soil organic matter as a result of soil compaction in heavy textured soils. The effect of soil compaction on C and N mineralization of the native SOM in light textured soils has been reported to be either positive, negative or no influence at all by different authors. Torbert and Wood (1992) found negative effects of soil compaction on microbial processes while Kaiser et al (1991) observed positive effects such as higher mineralization of C from added ^{14}C -labelled straw and van der Linden et al (1989) found no influence of compaction on C mineralization. Breland et al (1996) reported that, reduction of the total soil pore volume as a result of soil compaction increases the probability of anaerobic conditions. This may strongly inhibit degradation of lignocelluloses (Colberg, 1988), which has a major influence on the degradability of plant residues (Pinck et al, 1950). Strong et al, (2004) found no significant effect on C mineralization and soil biota after compacting the soil by applying 100Kpa pressure.

2.2.2 Soil pH and organic matter decomposition

Soil pH has strong influences on abiotic factors, such as carbon availability (Andersson et al, 2000), nutrient availability (Pietri et al, 2008), and the solubility of metals (Flis et al, 1993). Soil pH has been reported to have a profound effect on SOM preservation and decomposition (Motavalli et al, 1995). Many opposing ideas concerning the relationship between soil pH and SOM have been reported by several authors. Hardon (1936) found an increase of OC content in acid soils with a pH below 5 in forest soils while Spain (1990) observed a negative correlation between SOM and soil pH in tropical rainforest soils. Several researchers have reported an increase in C mineralization after soils being limed or reductions in carbon mineralization after acidification (Motavalli et al, 1995). Rousk et al (2009) reported an

increase of SOC content from 0.75 to 0.90% with an increase in pH from 4 to 4.5. In contrast, Oades (1988) predicted shorter C turnover times in acidic soils compared to calcareous soils. Motavalli et al (1995) in contrast suggested that acidic soil reduces the decomposition of freshly added organic materials. Condon et al 1993 suggested that these different observed effects of pH on organic matter decomposition may be attributed to the interaction of several factors in addition to pH that influence SOM formation and decomposition such as: soil temperature, moisture, texture, landscape position, ecosystem type, the physiochemical properties of soil organic fractions, P bioavailability and clay mineralogy.

2.2.3 Pore size distribution and carbon mineralization

The pore space of soil is composed of a continuum of pores ranging in size from micropores < 0.1 mm in diameter through to macropores >20 mm with an upper size limit in the order of centimeters (Baldock et al, 2000). All OM in soil is located within these pore spaces as discrete particles or molecules adsorbed onto soil particles. Strong et al, (2007) reported that, pore size distribution influences the location of microorganism, and the distribution of water and O₂ thus affecting OM decomposition. From their observations, they suggested two major reasons as to why pore size distribution plays an important regulatory role in decomposition and as to why OC may accumulate in different pore classes at different rates. First, they noted that decomposing organisms can gain access only to pores that can physically accommodate them. Secondly, pore size distribution determines where water resides in the pore system and since the microflora and fauna depend on this water for motility, water location has an important influence on biological activities in different pore classes. Pore size classes which ensure the adequate quantities of available water and oxygen optimize the processes of OM decomposition and mineralization (Baldock et al, 2000).

Pore size distribution also determines the air filled porosity and aeration status of the soil (Strong et al, 2004). Baldock et al (2000) reported that changes in the pore size distribution towards a greater proportion of large pores, such as noted in progressing from clay to sand, are accompanied by higher rates of C mineralization at equivalent values of air filled porosity. In their findings they also observed that reducing total porosity by compression induced reductions in C mineralization at all levels of air-filled porosity and shifted the air filled porosity at which C mineralization was maximized. Thomsen et al (1999) reported similar effects of pore size distribution, air filled porosity and volumetric water content on mineralization of C and N.

The pore size distribution also influences the ability of decomposer organisms to reach potential organic substrates (Baldock et al, 2000). Kilbertus, (1980) suggested that bacteria can only enter pores $>3 \mu\text{m}$. Regardless the actual size limit imposed, a lower limit of pore size into which organisms cannot enter does exist and decomposition of OM in these pores can only occur via diffusion of extracellular enzymes away from organisms towards a substrate and then diffusion of the products of enzyme reactions back to the organisms. The concept of exclusion, based on the physical size of the decomposer organisms, can be extended to predation of micro-organisms by soil fauna (Baldock et al, 2000). Van der Linden et al (1989) showed that protozoa and nematodes are excluded from pores $<5 \mu\text{m}$ and $<30 \mu\text{m}$, respectively. Therefore, OM residing in pores smaller than these diameters being in form of molecules, small particles, or bacterial or fungal tissues will not be susceptible to decomposition or predation by soil fauna. Baldock et al (2000) suggested that fungal proliferation in pore class 15-60 μm resulted in fast decomposition of label-derived C in this pore region. Strong et al 2007 observed a rapid decomposition of the added plant materials in soils with relative large volume of pores with neck diameters class from 15-60 μm and most slowly in soils with large volume of pores with neck diameters $< 4 \mu\text{m}$. Gregorich et al (1991) observed rapid OM decomposition in sandy soil than in clay soil and they attributed this to differences in soil texture and volume of small pores. Adu and Oades (1978) demonstrated that fungi had a clear advantage over bacteria in C decomposition in a fine sandy loamy but not in a clay soil because the fine sandy loamy has a higher proportion of pores that are easily accessible to fungi. Also in sandy soils fungal hyphae have the advantage to bridge considerable distance across air filled pores.

2.3 Soil pH and its effect on soil microbial community composition (Fungi/Bacteria ratio)

The soil microbial community is responsible for most nutrient transformations in soil, regenerating minerals that limit plant productivity. Fungi and bacteria are the major two groups dominating the microbial decomposer community, and they play an important role in decomposing SOM. There are potentially important differences between these two groups of organisms in their properties such as biomass elemental composition (De Ruiter et al, 1993), nutrient demand (Rousk et al, 2007), temperature dependence, and food web linkage (Moore et al, 2005). Hunt et al (1987) reported the general assumption to differentiate these two

decomposer groups with regard to their C: N ratio, where the C: N ratio of fungi is higher than that of bacteria. Therefore the carbon demand by fungi is higher due to their body structure and the N demand is higher for bacteria. Generally, human influences such as changes in nutrients inputs, climate change, and soil managements do have direct or indirect effects on Fungal and bacterial composition, with consequent impacts on soil functions (Rousk et al, 2009). These two groups of the microbial community can be distinguished through biomass based techniques such as microscopy and the use of biochemical markers including phospholipids fatty acids (PLFAs) (Zelles et al, 1999) and glucose amines (Appuhn et al, 2004), as well as DNA-based molecular techniques (Lauber et al, 2008).

Soil pH is one of the most influencing factors affecting the microbial community in the soil. In addition, soil pH may control biotic factors, such as the biomass composition of fungi and bacteria (Fierer et al, 2006), in both forest (Blagodatskaya et al, 1998) and agricultural soils (Arao, 1999). Rousk et al (2009) reported the fastest bacterial growth rate at pH above 7 and the growth rate tended to decline with decreasing pH by 80% from pH 7 to 4.5. The lowest bacteria growth rate was reported to be at pH 4 where the decrease reached 90%. A contrasting result was reported for the fungal growth rate, where the growth rate increased with a decrease in pH 8.3 to 4.5 and maximum fungal growth rate was reported at pH 4.5. The fungal to bacterial growth ratio observed to increase with decreasing pH from 8.3 to 4.5 and below pH 4.5 no clear trend was reported. Anderson and Domsch (1994) reported the increase of the Fungal to Bacterial ratio with decreasing soil pH. In an attempt to partition the relative contribution of Fungal and Bacteria decomposition activities, Bare et al (1990) reported the dominance of fungi decomposer group in the early stages of deciduous leaf litter decomposition. Persson et al (1989) reported that the initial mineralization process and particularly lignin degrading are dependent on carbon availability which was found to decrease at low pH. Therefore they suggested that fungi can better of sustain low pH compared to Bacteria (Swift et al, 1979) and also fungi are thought to use available carbon substrates more efficiently. Blagodatskaya et al (1998) reported an increase in respiratory activities of fungi under acidic conditions while that of bacteria decreased. Also Bewley and Parkinson (1985) reported that with increasing acidity; Bacterial respiratory contributions seemed to decline while the fungal respiratory contributions increased. Blagodatskaya et al (1998) suggested that soil pH is the major independent driving variable which controls the composition of the microbial community.

2.4 Spatial location of organic matter in the soil matrix

The soil matrix is an assemblage of mineral particles of various sizes, shapes, and chemical characteristics, together with organic materials, in various stages of decomposition and living soil populations. The matrix is characterized by its geometrical and chemical heterogeneity. Understanding the role of the soil matrix on decomposition of soil organic matter requires an assessment of how the soil matrix controls the availability of substrate to microorganisms (Gaillard et al, 1999). The composition and structure of the soil matrix are important factors determining the decomposition of OM in soil (Van Veen and Kuikman, 1990). The organization of the soil matrix can be well described through the framework of the soil pore system. Analysis of relationships between the volumes of different pore size classes and various soil properties can provide an understanding of soil organization within the framework of the pore system (Strong et al, 1993). This knowledge is important in understanding the mechanisms by which the soil matrix regulates biological processes, and is a primary point of attention in this master thesis.

OM in soils are distributed in the soil pore system (micro and macro pores) where microbial activities are active and its location within the soil matrix can affect its decomposition. Soil structure, especially the spatial distribution of OM within the organo-mineral matrix of soil, is considered to be one of the dominant controls over microbially mediated decomposition processes in terrestrial ecosystems (van Veen and Kuikman, 1990). Optical examination of the soil matrix revealed assemblages of OM and mineral particles in water-stable aggregates (Oades and Waters 1991). It has been shown that many aggregates about 100-200 μm in diameter have cores of plant debris. Encrustation of plant fragments by mineral particles was proposed as one of the mechanisms in microaggregate formation and stabilization (Oades and Waters 1991). SOM is found distributed as either well individualized particles, such as pieces of plant debris, or as amorphous OM. The differences in organic structure between free particulate OM, occluded particulate OM and amorphous OM represent the changes that OM undergoes during decomposition when it enters the soil where is enveloped in aggregates, and eventually is incorporated into microbial biomass and products and adsorbed to clay minerals (Golchin et al, 1994).

2.5 Soil structure and soil food web

2.5.1 Soil food web

The soil food web is the community of organisms living all or part of their lives in the soil. It describes a complex living system in the soil and how it interacts with the environment, plants, and animals. Soil life plays a major role in many natural processes that determine nutrient and water availability for plants. The energy needed for all food webs is generated by primary producers: the plants, lichens, moss, photosynthetic bacteria and algae that use sunlight to transform CO₂ from the atmosphere into carbohydrates. Most other organisms depend on these primary producers for their energy and nutrients; they are called consumers. The soil food web includes a wide variety of micro-organisms such as bacteria, viruses, fungi, protozoa and algae and they are part of soil organic matter. The soil food web also includes plant roots, insects, earthworms, and larger animals such as moles, mice and rabbits that spend part of their life in the soil. Food web studies have identified patterns of species interactions and material transfers between trophic levels and calculated the amount of biomass formed, and C excreted as inorganic (CO₂) and organic (fecal) materials (De Ruiter et al, 1993). Fig 1 below shows an example constructed food web for studying the OC and N mineralization in the soil (Moore et al, 1988). Microarthropods (collembola and Mites) and annelids were neglected because its contribution to mineralization was assumed insignificant (De Ruiter et al, 1993).

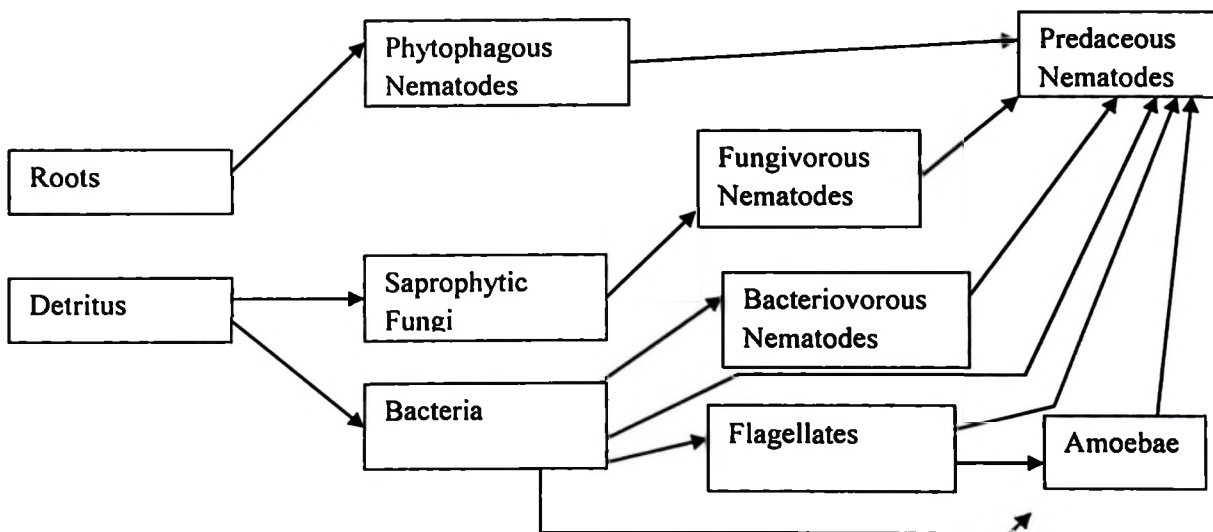


Figure 1 Diagram of soil food web on the grassland soil (Source: Jan Hassink, 1995)

2.5.2 Location of the soil organisms in the soil pore structure

The composition of the soil organisms depends on the food source and physical environment present (such as pore spaces which can physically accommodate them), moisture, aeration and soil pH. Thus they are not uniformly distributed in the soil. Organisms occur wherever OM occurs (Ingham, 2000). Therefore, soil organisms are concentrated around roots, in litter, on humus, on the surface of soil aggregates and in spaces between aggregates. For this reason, they are most prevalent in forested areas and cropping systems that leave a lot of biomass on the surface (Bot and Benites, 2005).

Strong et al (2004) reported that microorganisms in soil live in an ecosystem that is dominated by the solid particles. The spatial arrangement of the solid particles results in a complex and discontinuous pattern of pore spaces of various sizes and shapes that are more or less filled with water or air. The pore system can be viewed as the residence in which soil organisms are distributed as well as the environment in which interactions between organisms and their substrates are constrained. Pore-size distribution influences the distribution of soil water and air and thus the location of microorganisms in the soil. Strong et al (2004) suggested that, pore size distribution and its interaction with soil water led to a highly stratification of biogeography of organisms through the pore system which resulted in different rates of organic matter decomposition in pores of different sizes. Eliot et al (1980) postulated that, pores with neck diameter $< 0.2 \mu\text{m}$ are inaccessible to microorganisms. Using transmission electron microscopy, Kilbertus (1980) estimated the mean diameter of pores mainly colonized by bacteria to be $2 \mu\text{m}$, while no bacteria could be observed in pores below $0.8 \mu\text{m}$ diameter. Hassink et al (1993) found a positive correlation between the quantity of bacterial biomass and the soil volume made up with pores with pore neck size between 0.2 and $1.2 \mu\text{m}$ from which fauna predators like protozoa and nematodes with diameter from >5 and >15 are excluded (Foster, 1988). From this study, it was concluded that, pore size distribution not only controls the accessibility of microorganisms but also their fauna predation. The maximum diameter of the pores most frequently colonized by bacteria was estimated to be 2.5 to $9 \mu\text{m}$ for fine and coarse texture soils, respectively (Postma et al, 1989). Eliot et al (1980) reported that, pores of neck size diameter 0.8 - $6 \mu\text{m}$ are accessible mainly to Bacteria, pores $>5 \mu\text{m}$ are habitable by protozoa and pores of neck size diameter $>30 \mu\text{m}$ are needed by nematodes. Hassink et al (1993) also found a positive correlation between the quantity of nematode biomass and the soil volume made up with pores with pore neck size between 30 and $90 \mu\text{m}$. A fungal population dominates in sandy soils because

sandy soil has higher proportional of pores accessible by fungi thus their hyphae can bridge considerably across air filled pores (Strong et al, 2004). In their study using different soil types including sandy loam soil and after incubating soil samples for 4 and 90 days, the positive correlation was observed between fungal microbes and pore size class 15-60 μm . A negative correlation was also reported for the pore size class $< 15 \mu\text{m}$ and $> 300 \mu\text{m}$.

The effect of aggregate size on microbial activity depends on numerous factors. In some cases, microbial activity may depend on aggregate size (Mendes et al, 1999) and in others it may not (Schutter and Dick, 2002). Bacteria are often associated with clay and polysaccharides in microaggregates, resulting in lower microbial biomass in micro aggregates than macro aggregates (Lupwayi et al, 2001). Lower bacteria/fungi ratio in macroaggregates than in microaggregates suggest that bacterial activity may dominate in microaggregation while fungal activity dominates in macroaggregate formation (Tisdall and Oades, 1982). Increases in macroaggregates are associated with increase in fungal activities and fresh residues (Denef et al, 2001).

2.5.3 Impact of soil microorganisms on soil structure

Soil fauna and soil microorganisms are among the major factors that control aggregate formation and stabilization. Meso-fauna ($<1\text{-}2 \text{ mm}$) and macro-fauna ($<10\text{-}20 \text{ mm}$) contribute to soil structure by altering existing macropores and voids, or by creating new pores by penetrating the soil matrix (Brussaard and Juma, 1996). Sticky substances on the skin of earthworms and those produced by fungi and bacteria help bind particles together. Brown et al (2000) reported that earthworms can mediate soil aggregates through burrowing and cast formation. Earthworm casts are also more strongly aggregated than the surrounding soil as a result of the mixing of OM and the soil mineral material, as well as the intestinal mucus of the worm. Earthworms also stimulate microbial activity by increasing soil aeration and moisture, and by transporting litter into the ground where it becomes available to other soil fauna (Wardle, 1992). Recently, termites have received a well-deserved greater attention for their significant involvement in soil structural building. Mycorrhizal and saprophytic fungi are the most important soil microorganisms involved in the formation and stabilization of aggregates, but also bacteria can have profound influences on aggregation especially at the microscale (Six et al, 2004). The fungal mycelium has been described as a 'sticky string bag' because it entangles particles within the hyphae network and cements particles together through extracellular polysaccharide production (Waters and Oades, 1991). In contrast, the

production of mucilage by bacteria and fungi enhances only the formation of micro aggregates (Oades, 1993). In coarse textured sandy soils, aggregation is weakly related to microbial biomass and products (Degens and Sparling, 1996) because only the hyphae network is able to cross-link the abundant sand particles to form stable aggregates. In clayey soils, on the other hand, both bacteria and fungi and their products play a role in aggregation. Microbial influence is most pronounced in sandy soils where soil microorganisms produce readily available C-source for the rapid stabilization of aggregates (Kiem and Kandeler, 1997).

2.6 Stabilization of soil organic matter and protection against decomposition

SOM is a physical and chemical heterogeneous mixture of organic compounds of plant, animal and microbial origin, and has components at different stages of decomposition (Ratnayake et al, 2008). Baldock and Skjemstad (2000) reported that a continuum of decomposability of OM exists based on chemical structure; however, this continuum can be altered by interactions with minerals within matrices capable of stabilizing potentially labile OM against biological oxidation. Protection is not considered to equate to a permanent and complete removal of OC from decomposition, but rather to a reduced decomposition rate relative to similar unprotected materials. The stabilization of organic materials in soils by the soil matrix is a function of the chemical nature of the soil mineral fraction and the presence of multivalent cations, the presence of mineral surfaces capable of adsorbing organic materials, and the architecture of the soil matrix. The degree and amount of protection offered by each mechanism depends on the chemical and physical properties of the mineral matrix and the morphology and chemical structure of the OM. Each mineral matrix will have a unique and finite capacity to stabilize OM. Ratnayake et al (2008) suggested that the types of land use and soil are important factors controlling SOM storage in the soils. Their storage reflects the relative importance of different mechanisms of SOM stabilization. Several mechanisms have been proposed to contribute to the SOM stabilization in soils. The SOM can be (i) biochemically stabilized through the formation of recalcitrant SOM compounds, (ii) protected by intimate association with silt and clay particles, and (iii) physically stabilized through aggregate formation. This literature review discusses only the last protection mechanism.

2.6.1 Physical protection of soil organic matter

Location of soil organic matter within the soil matrix has an effect on its availability to decomposers or availability for decomposition. Strong et al, (2004) reported three ways on how soil pore size distribution plays as regulatory roles in organic matter decomposition and carbon accumulation on different pore classes in the soil. These are:

- Through its influence in the location of soil microorganisms in pores which can physically protect them.
- Through its influence in water distribution which promotes microbial motility and microbial activities in different soil pores.
- Through its influence in aeration thus affecting oxygen supply for microbial activities by enhancing aerobic conditions.

Next to the microporosity scale, physical protection of OM is relevant at aggregate scale. Thomsen et al (1999) suggested that, in undisturbed soils, organic matter residing between aggregates or in large pores may not decompose rapidly. Incorporation of OM into soil aggregates protects it from rapid decomposition and is one of the determinants of stability of OM in soils. Aggregates physically protect SOM by forming physical barriers between microbes and enzymes and their substrates and controlling food web interactions and consequently microbial turnover (Elliott and Coleman, 1988). Six et al (2002) reported that the physical protection of SOM exerted by aggregates is indicated by a positive influence of aggregation on the accumulation of SOM. In addition, cultivation has been found to result in a loss of SOM by breaking up the aggregates. Along with the accumulation of SOM by inclusion in aggregates, a qualitative change in the chemical structure of SOM has been found. The physical protection of SOM exerted by aggregates is predominantly at the microaggregate level. McCarthy et al (2008) postulated that the protection arises from spatial and kinetic constraints imposed on the ability of microbial exoenzymes to access and degrade the OM and on the delivery of hydrolysis products that can be assimilated by the microbes. From their study results it was suggested that the key process in the protection of OM in soil microaggregates is related to the architectural system of aggregate packing. Aggregates not only physically protect SOM but also influence the structure of microbial community (Hattori, 1988), limit oxygen diffusion (Sexstone et al, 1985), regulate water flow, determine nutrient adsorption and desorption (Linguist et al, 1997), and reduce run-off and erosion. Gale et al (2000) reported that soil microaggregate formation is crucial for the storage and stabilization of soil C in the long term. Six et al (2004) also found greater protection of soil

OM against decomposition within microaggregates than in macroaggregates. McCarthy et al (2008) proposed that pore-filling by OM, largely in submicron pores, is an important mechanism for protecting OM within microaggregates. Hassink et al (1992) found the physical protection mechanisms of organic materials to vary with soil texture. From this study it was observed that, in clay soils, physical protection was associated to location of organic materials in small pores. In sand soils, physical protection was through association of organic materials with clay particles and in loam soil, physical protection was through both two mechanisms.

2.7 Soil structure assessment

Several methods exist for the characterization of soil structure in the field and laboratory (Burke et al, 1986). This literature review is restricted only to three methods which can be easily determined in laboratory and for short time experiments. Soil structure assessment methods to be used in this study includes: Size fractionation of soil aggregates, characterization of the soil pore space using soil water retention curve, and examination of soil pore structure by CT.

2.7.1 Size fractionation of soil aggregates

The size, quantity and stability of soil aggregates recovered from soils reflect an environmental conditioning that includes factors which enhance the aggregation of soil particles such as OM amendments and factors that cause their disruption such as mechanical disturbance through soil cultivation. Therefore, measurements of stable aggregates depend on both the forces that bind particles together with the nature and magnitude of the disruptive forces applied (Beare and Bruce 1992). Wet sieving after slow wetting of the soil samples is one of the common methods used to determine aggregate size distribution. Dickson et al (1991) used four different wetting treatments (rapid wetting under vacuum, rapid wetting at atmospheric pressure, full dispersion by ultrasonic treatment and slow wetting) to compare aggregates distributions, from this experiment it was found that slow wetting at atmospheric pressure usually produced many water stable aggregates. Therefore slow wetting was concluded to be a more convenient method for determination of aggregates distribution in practice. Darbyshire et al (1992) however reported that, wet sieving method is not a convenient when isolating pre-existing micro-structures from soil to study the distribution of micro-organisms or organic matter. This was related to vigorous mechanical disturbances

during wet sieving. Ahmed and Oedes (1984) also reported the effect of vigorous shaking of soil slurries where the method was observed to result in destruction of major part of microbial biomass.

2.7.2 Soil Water Retention Curve

The soil water retention curve (SWRC) is an indicator of the pore size distribution and it is usually applied to soil pores smaller than 300 μm diameter (Darbyshire et al, 1992). SWRC is usually determined by measuring the moisture contents of soil samples equilibrated at a range of potentials, using suction tables and pressure plates instruments. The water retained at different matric potentials reflects the soil pore size distribution. Pore size distribution can be computed from the SWRC at each matric potential through the capillary equation $d = 0.3/h$ where d is the pore neck diameter (cm) and h is the matric potential (cm). The pore size distribution and continuity have an influence on soil water availability, gas diffusion and the movements of micro organisms (Scott et al, 1996)

Soil pore size distribution varies with soil texture since as particle size increases, the pore sizes also increases but the increase of pore sizes is not proportional to that of particle sizes. As soil particles increases, the porosity decreases thus soil with larger diameter particles (coarse textured) have a lower water content than soil with smaller diameter particles (fine textured). Soil water contents have an influence on microbial activities and in distribution of OM in soil pores. Zhuang et al (2008) reported that water content associated with the microaggregates structure is an important factor which is involved in various mechanisms of OM stabilization in the soils such as Physical protection. Due to relatively strong anaerobic environment which is often present in the micropores, pore-scale OM–water interactions, the biochemical transformation of soil OM in these pores can substantially be affected. On the other hand OM distribution in soil microstructures can also change soil pore connectivity, leading to changes in soil water flow among pores. All of these processes suggest that OM preservation closely interacts with water characteristics within soil micropores (Rawls et al, 2003).

2.7.3 X-Ray Computed Tomography

Computer tomography is a recent technique which can be used for soil structure analysis and it allows the investigation of spatial arrangement of mineral and organic components of the soil. X-ray CT is a non-destructive and non-invasive technique which has been successfully used for three dimension (3D) examination of soil. For many years, X-ray computed

tomography has been widely used as a medical diagnostical tool (Cnudde et al, 2006). Computerized tomography, originally developed in the medical field, and allows 2D or 3D reconstructions of internal features of an object. X-ray CT is an imaging technique that aims to reconstruct a slice through an object based on the attenuation of X-rays (Van Geet et al, 2000).

Historically, the first results of CT in soil science were reported by Petrovic et al, (1982) in an experiment that showed a linear relationship between soil bulk density and X-ray attenuation. Hainsworth and Aylmore (1983) subsequently utilized CT to study root-related water absorption processes. Over the intervening two decades, there has been considerable interest in the application of CT scanning in understanding many aspects of soil (Taina et al, 2008). Valuable information has been obtained by the application of CT for the description and quantitative measurements of soil structure elements especially of soil pores and pore neck features. CT has been used in visualization and quantification of soil porosity thus improving the knowledge of solution and chemical displacements (Perret et al, (1999). CT can be used for structural analysis specifically soil pores and and pore network for both undisturbed soil samples (Perret et al, 1999) and for packed soil cores (Anderson et al, 1990). Taina et al (2007) reported that, studies of soil hydro physical properties by using X-ray CT have shown accurate results when using soils with coarse-medium texture than clay soils due to shrinking-swelling processes problems of clay soils which causes changes in densities that cannot be separated from the changes caused by solutes infiltration itself.

2.7.3.1 Principles of X-ray Computed tomography

CT comprises three major parts, the X-ray source, the sample place and the detector where the X-rays are detected. The sample to be scanned is placed between the x-ray source and the detector, in a rotation movement in order to allow the attenuation of x-rays to be taken at different angles. The attenuation of the x-rays in an object depends on the bulk density and the elemental composition of the material. Nunan et al (2005) reported that, in the context of soil's pore space, water and mineral components such as quartz grains are characterized by low, intermediate and high X-ray densities respectively. The term density refers to the attenuation of X-rays within the sample and not to the physical density of the sample.

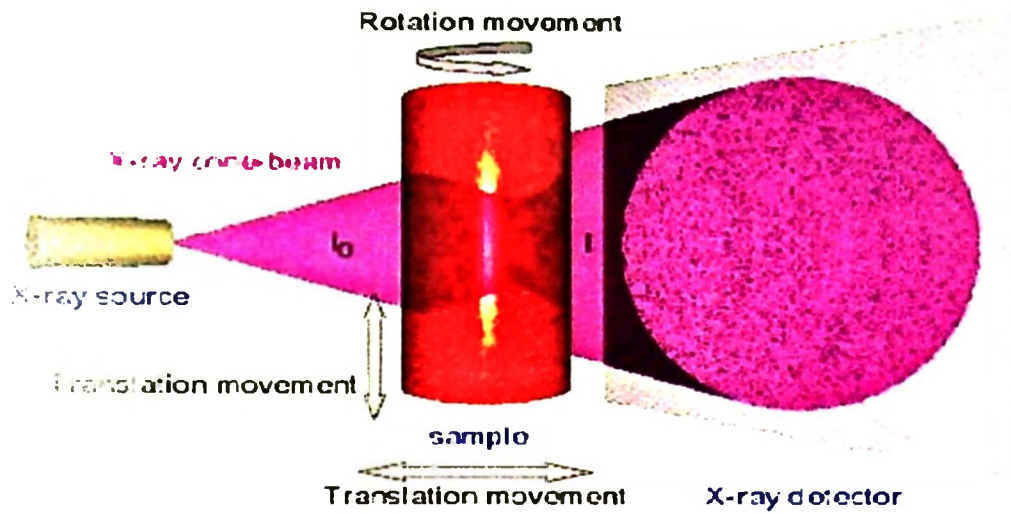


Figure 2 Diagram showing major components of CT (Source: Cnudde, 2005)

The theory of CT has been presented by numerous researchers in soil science and geology such as Cnudde et al, (2006). As the beam of X-ray radiation passes through materials, it experiences progressive attenuation due to interactions with the constituent atoms. This attenuation causes a gradual decrease in intensity through absorption and scattering (Fig.3). At energy levels typically encountered in CT scanners (<1MV), beam attenuation is primary a result of three mechanisms: Incoherent (compton) scatter, coherent scatter and photoelectric absorption (Simons et al, 1997). (i) Incoherent scatter involves the partial transfer of photon energy to an electron (exciting or ejecting it), and the scatter of lower energy photon (Kak and Slaney, 1988). This effect is influenced more by bulk density of the material (Simons et al, 1997). (ii) Coherent scatter is the redirection of the X-ray photon without a loss of energy. This effect is stronger at low energy level and for higher atomic numbers. (iii) Photoelectric absorption involves the total transfer of photon energy to the atom and the ejection of an electron (Kak and Slaney, 1988). This effect dominates at lower energy levels and is strongly dependent on effective atomic number (Mees et al, 2003). In general, the sensitivity to X-ray photon energy is lowest for incoherent scatter and highest for photoelectric absorption. The complexity of these attenuation mechanisms precludes simplistic interpretations of beam attenuation in heterogeneous material such as soil.

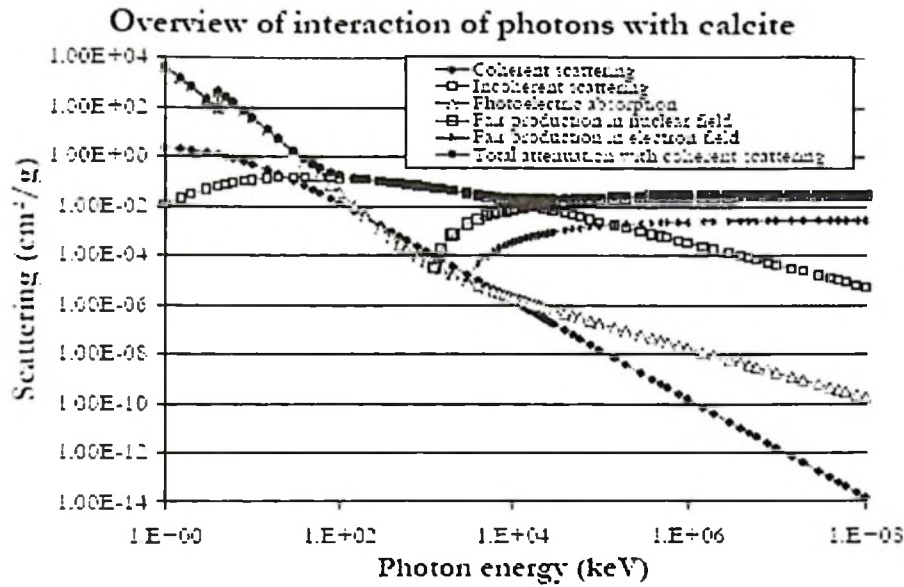


Figure 3 Overview of photon interaction with matter (calcite) depending on photon energy (Source: Cnudde, 2005)

For monochromatic X-ray sources the relationship in intensity between the incident and attenuated X-rays (respectively, I_0 and I) and h , the thickness of the object, may be expressed by Beer's law:

$$\frac{I}{I_0} = \exp(-\mu h) \quad (1)$$

Where μ is the linear attenuation coefficient at energy levels below 200 kV, linear attenuation is known to depend predominantly on two processes, namely photoelectric absorption and Compton scatter. This may be expressed as (Dyson, 1973)

$$\mu = \rho \left(a + b \frac{Z^{3.8}}{E^{3.2}} \right) \quad (2)$$

Where ρ is the bulk density of the material, Z is the bulk atomic number of the material, E is the X-ray energy and a and b the energy-dependent coefficients (Van Geet et al, 2000). Nunan et al (2005) reported that X-ray computed tomography works on the basis of materials exhibiting inherently different photon densities. Generally, CT images do reflect the relative physical density variations within the scanned object (Nunan et al, 2005). Using a dual energy configuration and 15 calibration materials (with densities varying from 0.5 to 2.7 Mg m⁻³ and thus relevant to soil systems), Wevers et al (2002) found that the density estimated from CT scans was very close to actual densities. Pedrotti et al (2003) found a highly linear

relation ($r^2=0.998$) between the attenuation coefficient of a range of materials (soils, water aluminum) and what they term tomographic units.

2.7.3 2 Application of CT in determination of pore size distribution and location of OM

Through the application of high resolution X-ray tomography in visualizing and quantifying 3-D structural variability for sewage sludge and biocides treated soil, Nunan et al (2005) observed no significant differences in soil porosity. In this study, soil porosity ranged from 27-47% for biocide and 22-41% for sewage sludge. These porosities were higher in comparison to what was observed by Adesodun et al, (2005) thus higher results were concluded to be attributed to major two reasons; Either due to higher resolution (grey scale value from 0-255 rather than HU with 10000 units) which was used which caused more porosity being visible or due to loosely aggregated materials of low density which may not have been distinguished from pore space. De Gryze et al (2005) used CT (resolution 13.4 μ m) to observe pore structure changes during decomposition of fresh residue in sandy loam and silt loam. In this study, from pore size distribution results, soil porosity in incubated aggregates of sandy loam was found to increase significantly and an extra porosity were in 27-67 μ m range. Total porosity was found to be higher in sandy loam than in silt loam due to an increase in void porosity (Pores occupied by air) after incubation while OM porosity (pores with OM) were the same for both soils. It was reported that, an increase in total porosity near OM residue was not attributed to the loss of OM but due to fungal hyphae pushing aside silt sized particles as it was found before by Dorioz et al (1993). Steven et al (2008) reported that CT, being a non destructive technique in combination with spatial analysis offers an opportunity to quantify directly the internal architecture of soil in three dimensions. Therefore the technique helps in exploring the spatial organization of soil and particularly of the spatial location of OM and microorganisms in the soil matrix (Young et al, 2001).



CHAPTER 3: MATERIALS AND METHODS

3.1 General experimental set up

Two lab incubation experiments were set up to investigate the relationship between soil pore structure (assessed for X-ray CT images) and the decomposition process (assessed from CO₂ evolution measurements). Essentially, repacked soil columns were included after artificial manipulations of soil structure and /or substrate addition.

POM was removed by a combination of sieving and recombining the soil. This allows better assessment of C mineralization from the added substrates. A first experiment was designed to investigate how artificial operations (compaction, change in texture, acidification, OM application) succeed in manipulating either the pore size distribution, the decomposer group community (assessed by the fungal:bacteria ratio) or both. Soil samples were incubated in larger tubes and the evolving CO₂ gas from respiration was analyzed by NaOH captation method.

A second incubation experiment was set up to follow C mineralization of added C- sources in artificially manipulated soil with differing pore size distribution and decomposer community. These samples were scanned after incubation by X-ray CT and following image processing the pore size distribution was assessed. Correlation analysis was used to investigate the relation between pore size distribution and C mineralization.

3.2 Soil

The soil used in this study was a sandy loam from Lendelede (arable cropping field). A 20 kg soil sample was taken from 0-30 cm soil layer by spade. The soil was characterized by 0.797% OC, 0.061% N, pH_{H2O}, 6.3 (measured with a ratio of 1:5), and 1% soil moisture content (for air dried soil)

3.2.1 Soil sample preparation

The soil was bulk sieved to separate sand fractions from silt and clay fractions through dry sieving on 2000, 500, 200 and 53 µm sieves. The sand fractions were wet sieved again after complete particle dispersion by shaking soil with sodium metaphosphate (SOIL: SMP ratio of 1: 3) on 500, 200 and 53 µm sieves. The >500 and 200-500 µm fractions were combined. In this way three soil fractions were obtained namely, coarse sand (200-2000 µm), fine sand

(53-200 μm) and silt & clay (<53 μm). The coarse and fine sand fractions were placed in a muffle furnace at 500°C for 5 hours to remove the (particulate) organic matter. An artificial soil with no particulate organic matter was reconstructed by mixing coarse sand fraction (200-500 μm diameter), fine sand fraction (53-200 μm diameter) and silt & clay fractions (<53 μm) to a ratio of 10:40:50 resulting to a soil with 50% sand, 50% silt and clay fractions (model Sandy loam). The reconstructed soil had 0.035%N and 0.448%C, pH 6.3, and bulk density 1.3 g cm^{-3} .

3.3 First incubation experiment

A hundred gram of the reconstructed soil was filled in incubation tubes with a radius of 2.25 cm and treated with eight different treatments (Fig 4) next to control treatments with pH 6.3, bulk density 1.3 g cm^{-3} and a coarse sand: fine sand: silt & clay ratio of 10:40:50. A part of the tubes (6) were filled with different ratios of coarse sand, fine sand and silt & clay fractions, namely, 15:50:35 and 20:60:20) to simulate differences in particle and pore size distribution. Three tubes were compacted to a height of 3.9 cm (instead of 4.8 cm for a bulk density of 1.3 g cm^{-3}) to simulate bulk density of 1.6 g cm^{-3} . Six tubes were filled with soil mixed with either 0.25g of saw dust or 0.25g of grass (equivalent to 9.75 t ha^{-1}) to simulate differences in decomposition of contrasting organic matter types. The organic materials were grinded and sieved in 200 μm sieve to obtain fine fractions. A last group of 6 tubes were prepared to simulate different pH ranges at pH 5.3 and 4.3. The pH was adjusted (lowered) by applying 6 and 15ml of 0.01M HCl with concomitant lower application of distilled water. For each treatment, the control was included to observe the carbon mineralization from native soil organic matter and under 'non manipulated' soil conditions. Soil water content in all tubes were adjusted to 50% water field pore space (WFPS) taking into account 1% moisture content in the air dried soil.

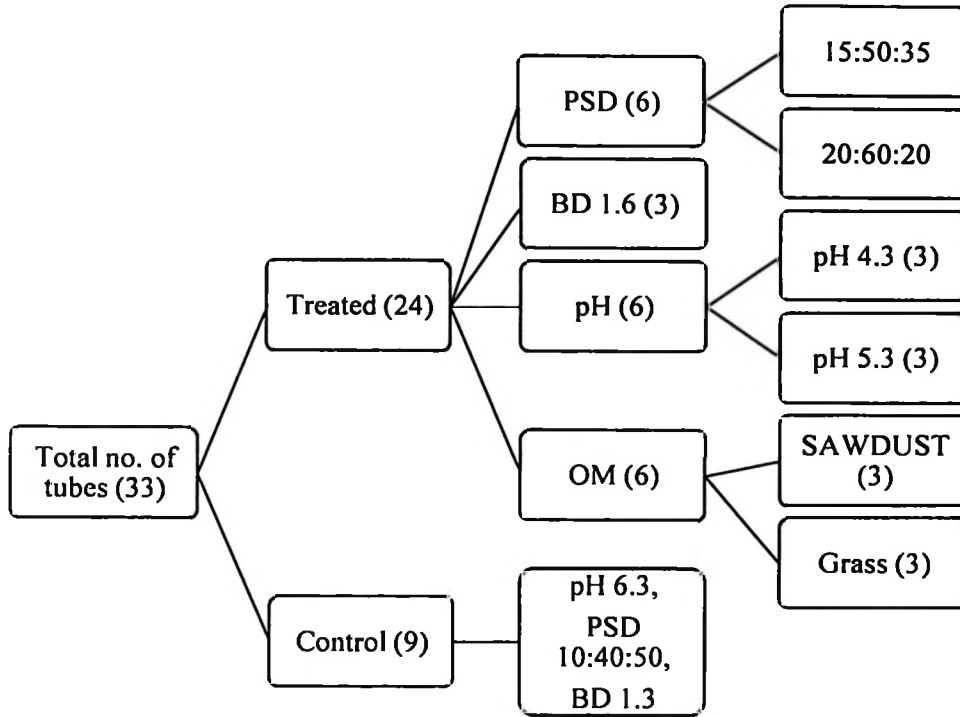


Figure 4 Experimental set up for the first incubation experiment including artificial soil manipulations (compactions, acidification, substrate addition and artificial change in soil texture).

3.3.1 C mineralization

All prepared incubation tubes were then placed in glass jars with an inner diameter of 10.3 cm. The initial weights of jars together with soil filled tubes were recorded. Small vials containing 15ml of 1M NaOH solution were placed in jars to trap the evolved CO₂. The jars were closed with air tight seals and incubated at 20°C. The samples were taken after 1, 4, 8, 11, 15, 19, 23, 26 and 33 days by removing the NaOH vials. The amount of the evolved CO₂ was measured by titrating the remaining NaOH with 1M HCl to a pH of 8.3 in the presence of BaCl₂ (Anderson 1982). The net amount of CO₂ produced from the different treatments was determined from the difference between CO₂ produced from treated soils and the CO₂ evolved from the corresponding controls. After removal of vials, the glass jars were left open for at least half an hour to allow replenishment of O₂. Soil moisture content was adjusted after every two weeks, the fresh vials containing NaOH were added and the jars were sealed again to continue the C mineralization measurements.

3.3.2 Determination of microbial biomass carbon

Microbial biomass carbon (MBC) was determined at the end of the 5th week of incubation. MBC was determined using the fumigation-extraction technique (Vance et al, 1987). Ethanol-free CHCl₃ was used for fumigation. Chloroform is an effective biocide and does not solubilize SOM. Both fumigated soil and unfumigated controls (20 g) were extracted with 40 ml of 0.5 M K₂SO₄. Extracts were stored in a freezer at -18°C for later analysis. The soil (MBC) was calculated from the difference between OC in the extract of fumigated soil and the unfumigated soil (Sparling et al, 1996). To correct for the incomplete release and extraction of soil MBC an extraction efficiency kEC value of 0.45 according to Joergensen, (1996) was used. The moisture content from each soil sample was determined from 10 g of soil using a standard procedure.

3.3.3 Determination of aggregate size distribution

Aggregate size distribution was determined by wet sieving method. Thirty grams of soil after incubation experiment was weighed into petridishes, 10 ml of water was added below the cloth covering the petridishes, and then the sample was kept in a fridge overnight to moisten the soil through capillary rise. There after the soil was wet sieved through a set of sieves with dimensions 500µm, 200µm and 53µm to collect coarse and fine sand fractions and soil aggregates of this size. The sieves were then gently shaken up and down under water at a constant speed (automatically controlled) for five minutes according to De Leenheer and De Boodt (1959). After sieving the wet sand fractions and aggregates remained in each sieve was removed by washing them into Aluminum cups. The aggregates were allowed to settle in Aluminum cups for 15 minutes then the cup was placed into oven (100°C) to dry. After drying, the sand fractions and aggregates were allowed to cool and then each sand fractions and aggregate sizes were weighed.

The same procedure was repeated for the original constructed soil (soil not incubated) after dispersion with sodium hexa meta phosphate to determine the amount of sand fractions with diameter >500 µm, 500-200 µm, 200-53 µm. Amount of aggregates formed after incubation was determined by subtracting sand fractions of original constructed soil from sand fractions and soil aggregates of the same sizes from the incubated soil samples.

3.3.4 Determination of Phospholipids Fatty Acid (For analysis of Fungi to Bacteria ratio)

Phospholipids in the soil were extracted using a modified Bligh and Dyer technique (Bligh and Dyer, 1959). Four gram freeze-dried soil was weighed in glass tubes. Lipids in the soil samples were extracted twice by adding 3.6 ml phosphate buffer pH 7.0, 4 ml chloroform and 8 ml methanol. Suspensions were shaken for 1 h and afterwards centrifuged for 10 min (2500 rev min⁻¹). The supernatants of both extraction cycles were collected in reparatory funnels and 8 ml phosphate buffer and 8 ml chloroform were added. Samples were left overnight for phase separation. The next day, the lipid layer was transferred to new tubes, dried under N₂ and redissolved in chloroform. The lipid extracts were separated into neutral, glyco- and phospholipids by chloroform, acetone and methanol respectively using silica columns (Chromabond, Macherey-Nagel GmbH, Düren, Germany). Chloroform and methanol fractions were dried under N₂. The dried lipids were then dissolved in 1 ml methanol: toluene (1:1 vol: vol) and 1 ml 0.2 M methanolic KOH. Samples were incubated at 35°C for 15 min to allow transesterification to methyl esters. After cooling to room temperature, 2 ml hexane: chloroform (4:1 vol: vol), 1 ml 1 M acetic acid and 2 ml water were added. After vortexing, the samples were centrifuged for 5 min (2000 rev min⁻¹). The hexane layer, containing the methylated fatty acids, was transferred to pointed tubes. The aqueous phase was washed twice with hexane: chloroform. The combined hexane phase was dried under N₂. The fatty-acid methyl esters were finally dissolved in 0.3 ml of hexane containing methyl nonadecanoate fatty acid (C19:0) as internal standard. PLFAs was determined by GC-MS on a Thermo Focus GC combined with a Thermo DSQ quadrupole MS (Thermo Fisher Scientific Inc., Waltham, USA) in electron ionization mode. Samples were injected on a Restek capillary column Rt-2560 (100 m x 0.25 mm i.d., 0.2 µm film thickness; Restek, Bellefonte, USA).

3.3.5 Determination of Soil Water Retention Curve

3.3.5.1 Soil preparation.

A parallel set of soil tubes was prepared for determination of the soil water retention curve to assess the effect of the artificial soil treatments on the soil pore distribution. A similar experimental set up as in the first incubation experiment was designed. However, in this experiment everything was prepared with 50g of soil in smaller tubes with an open bottom

sealed with nylon cloth to allow for direct placement on the sand box after incubation (Fig 5) and only two replicates were prepared per treatment. Fifty grams of the reconstructed soil was filled in the plastic tubes with soil column height of 4.5 and 3.7 for bulk density 1.3 and 1.6 g cm⁻³, respectively. The soil was treated with eight different treatments next to control treatments with pH 6.3, bulk density 1.3 g cm⁻³ and a coarse sand: fine sand: silt&clay (CS: FS: S&C) ratio of 10:40:50. A part of tubes (4) were filled with different ratios of CS: FS: S&C fractions namely, 15:50:35 and 20:60:20) to simulate differences in particle and pore size distribution. Two tubes were compacted to a height of 3.7 cm (instead of 4.5 cm for a bulk density of 1.3 g cm⁻³) to simulate bulk density of 1.6 g cm⁻³. Four tubes were filled with soil mixed with either 0.125g of saw dust or 0.125g of grass (equivalent to 9.75 t ha⁻¹) to simulate the differences in decomposition of contrasting organic matter types. A last group of 4 tubes were prepared to simulate different pH ranges at pH 5.3 and 4.3. The pH was adjusted (lowered) by applying 3 and 7.5ml of 0.01M HCl with concomitant lower application of distilled water. Then the samples were incubated for 5 weeks to allow similar soil structure formation as in the C mineralization experiment.



Figure 5 An image showing the type of soil filled tubes used in the determination of the SWRC

3.3.5.2 Soil Water Retention Curves

The soil samples' soil water retention curve (SWRC) was constructed by measuring soil water content at eight soil-matric potentials (-10, -30, -60, -100, -200 cm, 1/3bar, 1bar and 15bar) using the stabilized soil samples following a procedure described in Cornelis et al (2005). For the pressure potentials ranging from -1 to -10 kPa (-10 to -100 cm), the sand box apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) was used. Each tube was placed on the sandbox in 1 mm of water and gently pressed downward to create a good contact between the sample and the sand. To saturate the samples by capillary rise, the water level on top of the sand was raised until 2.5 cm (halfway the sample height). Once the samples were saturated, suction was applied by adjusting the suction regulator of the sandbox apparatus. After having reached equilibrium between the applied pressure and the quantity of water in the sample, the samples were removed from the sandbox, weighed and placed back on the sandbox, and the suction applied on the sample was increased. After having determined the sample weight at -10 kPa (-100 cm), a subsample was taken, weighed and placed in the oven at 105°C for 24 h, and weighed again to determine the water contents at pressures between -1 and -10 kPa. This allowed to calculate the bulk density, the sandbox was thus used to determine five (θ , ψ) data pairs on one single soil sample.

This sample was further divided into undisturbed subsamples using sharpened steel 20-cm³ cylinders. These undisturbed subsamples were used to determine water content at -20, -33 - 100 and -1500 kPa using pressure chambers (Soil moisture Equipment, Santa Barbara, CA). After having obtained equilibrium between the applied pressure and the quantity of water in the sample, the samples were weighed and placed in the oven at 105°C for 24 h. Then they were cooled and weighed again to calculate the water content. van Genuchten (1980) model was used to fit the SWRCs. Curve fitting was performed using RETC version 6.0 software (van Genuchten et al, 1991). It uses, a non-linear least-squares approach based on Marquardt's maximum neighborhood method (Marquardt, 1963). Pore size distribution was determined from water retention curve through the application of capillary equation $D = 0.3/h$ where d is the pore neck diameter in cm, and h is the matric potential in cm.

3.3.6 Determination of soil pH

The pH_{H_2O} was determined from the reconstructed soil. To determine the pH, 10g of the reconstructed soil was weighed in 50ml glass beaker. 50ml of water was added and stirred

with a glass rod; the mixture was left to stand for 18hrs. Thereafter, the suspension was stirred again, and then $\text{pH}_{\text{H}_2\text{O}}$ was measured with a glass pH-electrode

3.4 Second incubation experiment

The reconstructed soil (1.02g) was filled in small incubation tubes with dimensions of 1cm diameter by 1cm height making a soil column height of 0.99 and 0.81cm for bulk density of 1.3 and 1.6 g cm^{-1} respectively. The soil was treated (Fig 6) by a combination of two treatments next to control treatments with bulk density 1.3, 1.6 g cm^{-1} and texture with CS: FS: S&C ratio of 20:60:20. A part of tubes (6) tubes with BD 1.3 g cm^{-1} were mixed with either 0.0027g of grass material or 0.0027g of saw dust (equivalent to 10.3 t ha^{-1}) to simulate the differences in decomposition of contrasting organic matter types for non compacted soil. Another group of tubes (6) were compacted to BD 1.6 g cm^{-1} and mixed with the same type and ratio of OM as above to simulate the differences in decomposition of contrasting organic matter types at this level of compaction. A last group of tubes (6) with CS: FS: S&C of 20:60:20 were also mixed with the same type and same ratio of OM as above, to simulate the differences in decomposition of contrasting organic matter types at this artificially changed soil texture. Soil water content in all tubes were adjusted to 50% water field pore space (WFPS) taking into account 1% moisture content of the air dried soil. All prepared incubation tubes were weighed after being covered with parafilm in order to control moisture loss by evaporation during incubation period, through timely addition of water with a 10ml syringe.

3.4 1 Carbon mineralization by Gas Chromatography method.

In the second incubation experiment CO_2 respiration was not measured by the NaOH captation method (used in the first experiment) because it is not sensitive enough for measurement of small quantities of CO_2 , expectable for the small soil cores used. Instead, CO_2 respired by the soil was deduced from timely determinations of the CO_2 concentration in the

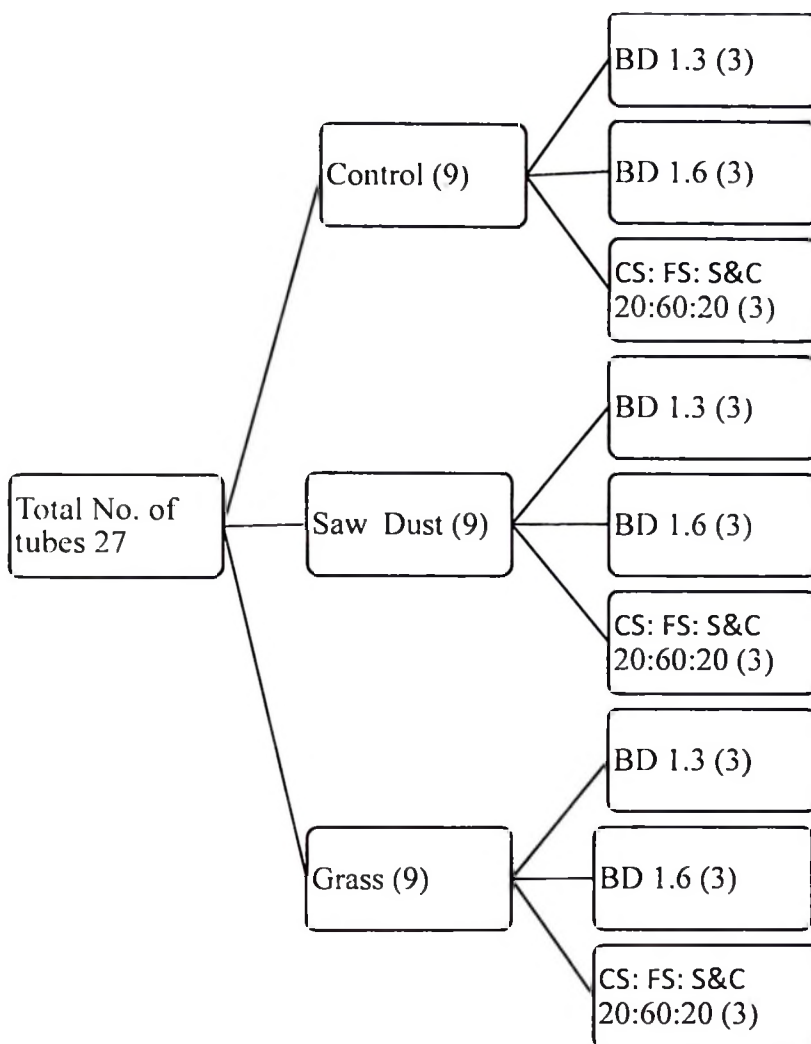


Figure 6 An experimental set up for the second incubation experiment

closed containers by sub sampling the headspace. Either a 50 or 250 μl syringe was filled with the headspace air through a septum and this gas sample was injected manually into a GC. Analysis of the gas samples was done by a Thermo Electron Trace GC Ultra gas chromatograph with a split/splitless injector; ‘CP – Pora Bond Q Fused Silica’ capillary column (length 25 m internal diameter 0.53 mm) and Electron Capture Detector (ECD) with a ^{63}Ni β -source. Table 1 gives specifications of the GC settings optimized for CO_2 measurement. The carrier gas was N_2 alphasaz 2 quality. Further specifications are given in Table 1.

Table 1: Settings of the GC for CO₂ measurement

Component	Parameter	Value
Oven	Temperature	30 °C; constant
	Hold time	3 min
Right inlet	Inlet	200 °C; splitflow 19 ml/min
Right carrier	Mode	Programmed pressure
	Initial ramp	29 kPa; 1,45 min
	Ramp 1	18 kPa; 1,46 min
	Preceding decrease	120 kPa/min
Right detector	Base temperature	240 °C
	ECD temperature	250 °C
	Reference current	1 nA
	Pulse amplitude	50 V
	Pulse width	1 μs
	Flow makeup	22
Run table	Prep run	Right detector / autozero
	At 1,45 min	External event #1 / off

A standard curve describing the linear relation between peak area and quantity of CO₂ (in μg CO₂) was established by injecting 0, 10, 20, 30, 40 and 50 μl of a 3% CO₂ (in He) standard. From the calculated quantity of CO₂ in the syringe, the quantity accumulated in the headspace was calculated assuming a 30 cm³ headspace. CO₂ respiration during opening of the containers was estimated by multiplying the time open with the average respiration rate calculated from the closed container at the same and previous samplings. CO₂ in the headspaces were analyzed at 1, 3, 5, 7, 15, 21, 29, and 35 days after initialization of the incubation experiment. All measurements were corrected for ambient CO₂ present in the air by including two replicate empty containers.

3.4.2 Determination of total porosity and pore size distribution by X-ray computed tomography

X-ray computed tomography

18 Small soil columns (height 1cm, inner Ø 1cm) repacked with the sandy loam textured soil were scanned with a state of-the-art high resolution x-ray tomography set-up at the Ghent University Centre for X-ray Tomography (UGCT, www.UGCT.Ugent.be). The experimental conditions for the micro-focus CT scans consisted of a directional target microfocus X-ray tube operated at 100kV and 80 μ A (8W), an amorphous silicium flatpanel detector (Varian Paxscan 2520) with 1400ms exposure time per projection and 1400 projections per scan of 40 minutes. The raw data was reconstructed with the in-house developed reconstruction software Octopus to a dataset of 1500x1500x1500 cubic voxels with 10 μ m voxel pitch for each soil column.

3.4.2.1 Image processing

The scanned images were analyzed for total porosity and pore size distribution using Morpho+ (Christmas edition, UGCT). For the calculation of pore size distribution, an equivalent diameter was calculated which is the diameter of a circular sphere having the same volume as the pore. All 18 images were processed identically to allow comparison of the calculated pore size distributions. Figure 7 shows the procedure followed during image processing.

First, a volume of interest (VOI) was selected to decrease image processing time. These VOI of 350x300x300 voxels were filtered with two times a median (26x26) and a bilateral (2,2) filter. Both filters reduce noise by replacing each voxel value by the average of nearby voxels, but the bilateral filter also preserves the edges of objects in the image. The objective of filtering images is to simplify the second step, segmentation. Segmentation refers to the process of partitioning a digital image into multiple segments (sets of voxels) which receive the same visual characteristics. It assigns a label to every voxel in the image, separating the background features from the ones that are interesting to analyze. Based on the histogram, a threshold values between 23 and 43 grey value were chosen to define pore space apart from the soil matrix. This second step of the image processing results in a binary image, where

background voxels turn black (value 0) and foreground voxels of interest turn white (value 1) (Fig 7). All further image processing steps are applied to these white foreground voxels.

The third step is labeling, which assigns a label (=number) to every discrete object. Because a large part of the pore volume in soil is interconnected, this step results in 1 object with a large volume, and a number of small objects. In reality, the pore matrix does consist of interconnected pores, but they are connected through narrow pore necks. By determining the location of these pores necks, this large object was divided into a number of objects. This division was done in steps 4 and 5 (Fig 7). Step 4 calculates a distance map which labels each voxel of an image with the distance to the nearest edge of its object. This distance map is used as input for step 5, a watershed based separation. The latter uses the distance values as topographical information, where all the minima are called catchment basins and all the lines that separate these basins are called watersheds. The catchment basins (=minima) are in this case the relevant objects, where the watersheds (=maxima) are the border of the objects. Step 5 results in a labeled image, where each soil pore is represented by a different label number (Fig 7) which allows analysis of these objects, step 6. From the obtained data, a distribution

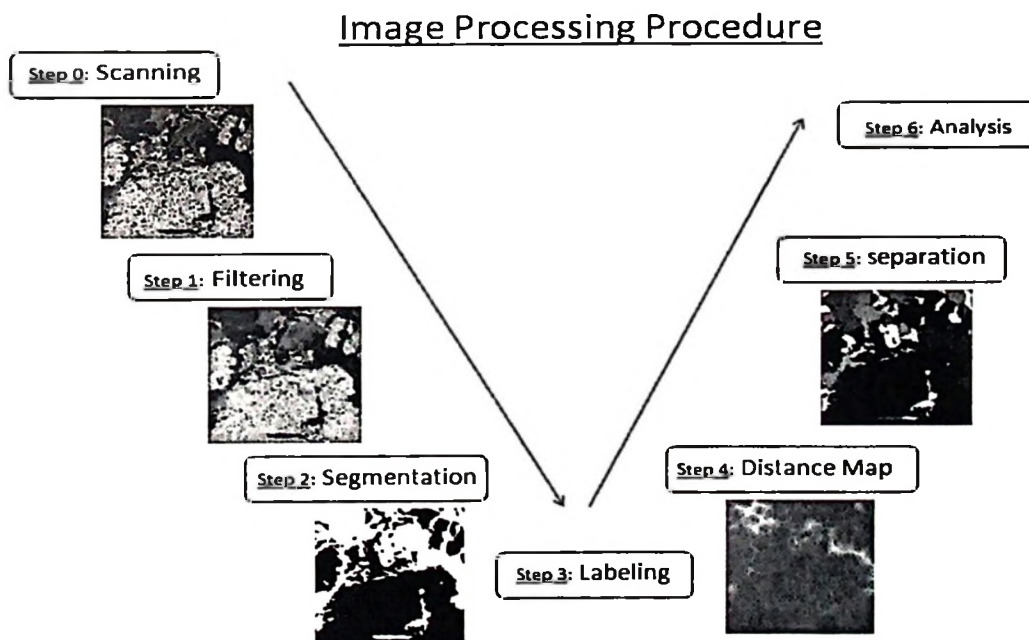


Figure 7 Diagram showing image processing procedure

of voxels according to equivalent sphere diameter was used to calculate the pore size distribution. The total pore volume (%) was calculated from the ratio of the total volume (μm^3) of the equivalent sphere diameter and total volume (μm^3) of the scanned volume. The volume of the specific pore class (%) was calculated from the ratio of volume of equivalent sphere diameter for a specific class and total volume (μm^3) of the equivalent sphere diameter.

CHAPTER 4: RESULTS

4.1 First Incubation experiment

A first experiment was aimed to investigate the influence of artificial manipulations on soil pore structure and microbial decomposer community. The treatments used under this experiment were compaction, artificial change in soil texture, pH and OM application (see 3.3). The following results (4.1.1 – 4.1.4) describe the effect of these treatments on soil pore structure and the microbial decomposer community.

4.1.1 pF-Curve and aggregate size distribution

Wet sieving by a nest of sieves at mesh sizes 500, 200 and 53 μm was employed to investigate the effect of artificial manipulations of the soil on soil aggregate size distribution, which indicates changes in soil (pore) structure. Results from the derived aggregate distribution as assessed by wet sieving method showed only very weak soil aggregation on all treated soils (Fig 8). Probably, as a result of the sandy texture and very low OC (0.191-0.448 %), aggregate stability was only weak and most macro-aggregates were not water stable. Consequently, the wet sieving method was unable to deliver any usable information on aggregates and pore size distribution.

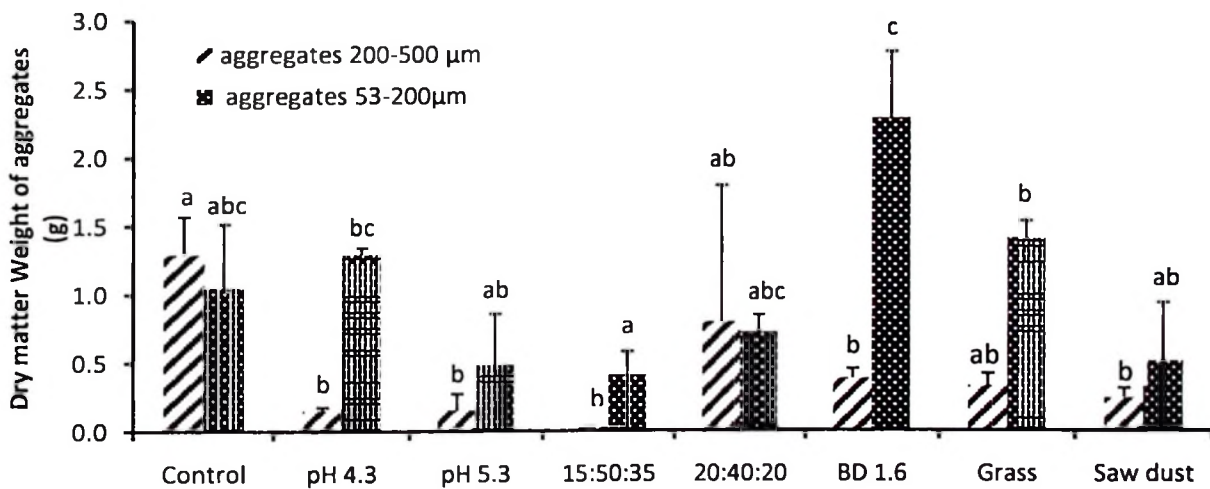


Figure 8 Soil micro aggregates dry matter distribution after 5 weeks soil incubation period. 15:50:50, 20:60:20 are the ratio of CS: FS: S&C. (Treatments presented by the same letter are not statistically different according to Duncan's LSD post hoc test) (30g of soil sample weighed)

Fig 9, 10 and 11 show the pore size distribution for all treatments as derived from water retention curves. The pore size distribution according to the pore neck diameter was

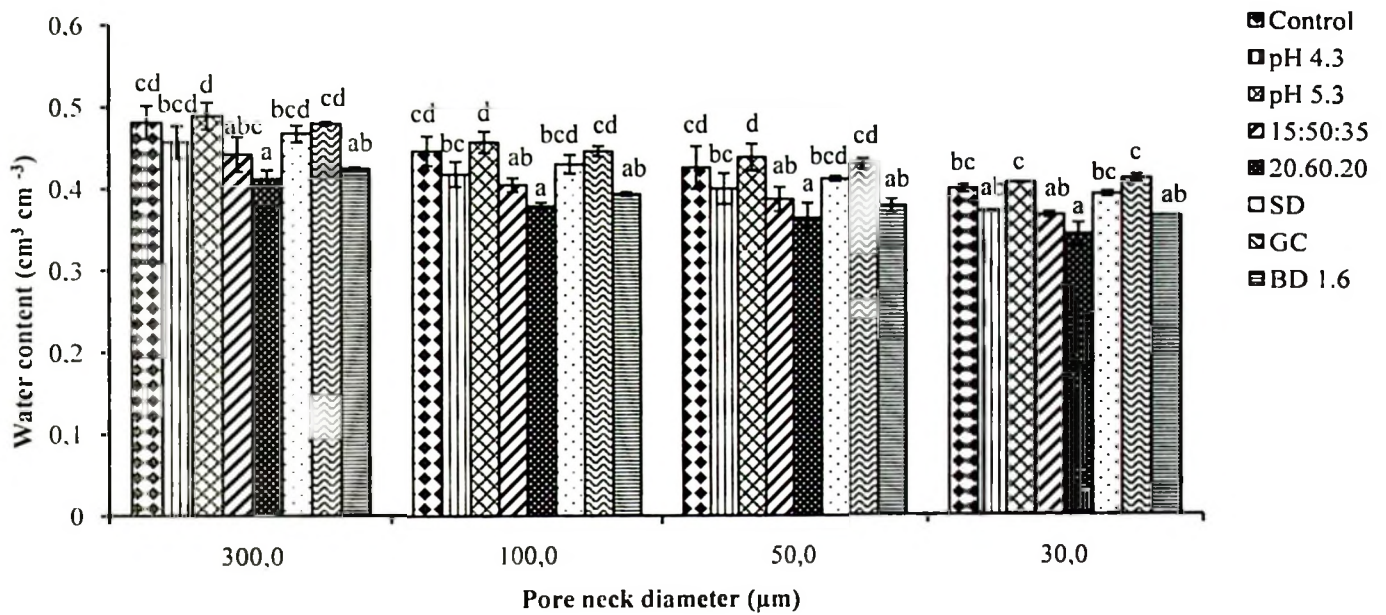


Figure 9 Soil water content for all treatments at every given pore neck diameter size. (300-30 µm). (Treatments presented by the same letter are not statistically different according to Duncan's LSD post hoc test) (15:50:50, 20:60:20 are the ratio of CS: FS: S&C, GC = Grass, SD = Sawdust)

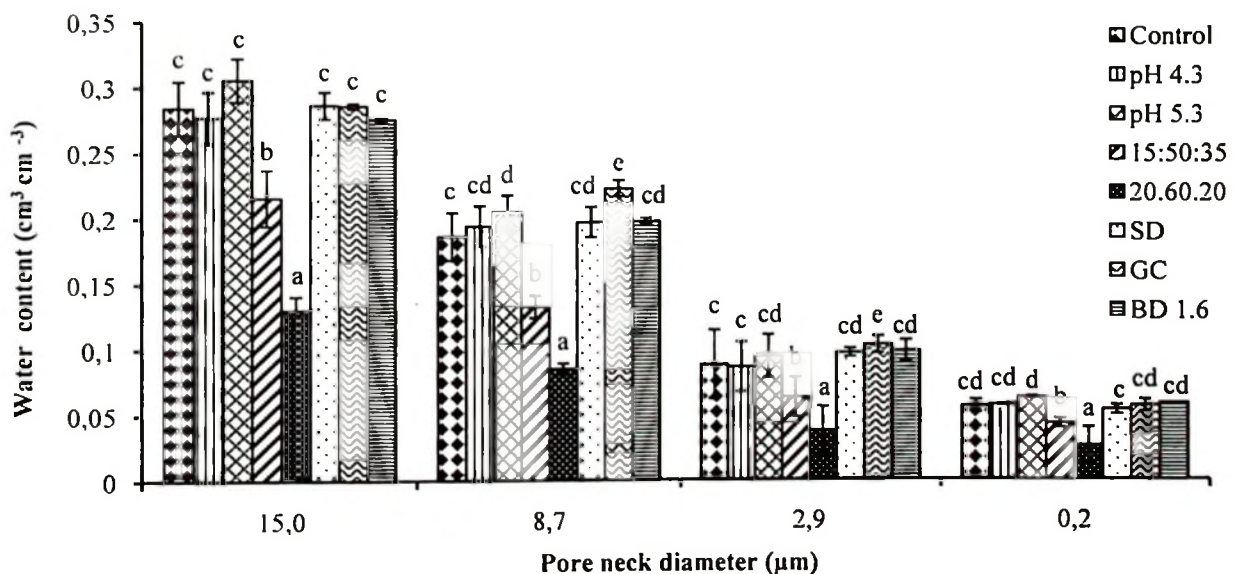


Figure 10 Soil water content for all treatments at every given pore neck diameter size (15-0.2 µm). (Treatments presented by the same letter are not statistically different according to Duncan's LSD post hoc test)

4.1.2 Microbial biomass and Carbon mineralization

4.1.2.1 Modeling OC mineralization

Fig 12 shows graphs from the measured C mineralization as a function of incubation time ($C(t)$ in $\text{mg C } 100 \text{ g}^{-1}$) and a fitted parallel first plus zero order mineralization kinetics model ($C(t) = CA_f(1 - \text{EXP}(-k_f * t)) + k_s * t$) for treatments Compaction (bulk density 1.6 g cm^{-3} vs. bulk density 1.3 g cm^{-3} (Control), pH (pH 4.3 and 5.3 vs. pH 6.3-control), OM application (Sawdust and Grass vs. no application-Control) and artificial change in soil texture, CS:FS:S&C of 20:60:20 and 15:50:35 vs. 10:40:50-Control). CA_f is the amount of fast decomposable C present, k_f and k_s are the mineralization rates of the fast and slow decomposable OC pools respectively. In general the R^2 values for all the treatments were close to 1, implying that the selected model could describe the mineralization process appropriately. Within the five 5 weeks incubation, the course of the cumulative mineralization mainly followed a linear course starting at approximately 4-5 weeks.

Carbon mineralization within the 5-weeks period from SOM decreased in the compacted soil (bulk density 1.6 g cm^{-3}) from $85.72 \text{ (mg C kg}^{-1}\text{)}$ at bulk density 1.3 g cm^{-3} (control) to $65.38 \text{ mg C kg}^{-1}$ (Table 2), equivalent to a 23.7% decrease. A similar decrease in the rate of C mineralization was observed with acidification to pH 4.3 (26.3% decrease) but not at pH 5.3, where C mineralization was found to be almost the same as in the Control soil (pH 6.3). For artificial texture treatments, C mineralization found to decrease strongly with increasing sand fractions (from 10:40:50-control to 20:60:20 coarse sand: fine sand: silt and clay fractions) where C mineralization decreased from $85.72 \text{ (mg C } 100\text{g}^{-1}\text{)}$ for control (CS: FS: S&C 10:40:50) to $31.54 \text{ mg (C } 100\text{g}^{-1}\text{)}$ equivalent to a 63.2% decrease (Table 2). It should be noted that the amount of initial SOC in these treatments is different because of varying amount of the silt and clay sized fraction, with the removal of all sand sized OM. Highest C mineralization was observed in soil applied with grass, followed by the saw dust treatment relative to the control, with a trend of 519.75, 154.00, and $85.72 \text{ mg C kg}^{-1}$ for the grass, Sawdust and control treatments, respectively. But relative mineralization should be considered in these treatments because the mineralization was not only from the SOM but also from the added OM.

Parameters of the parallel first and zero order kinetic model fitted to the cumulative C mineralization data are given in Table 2. The values of the parameters varied between

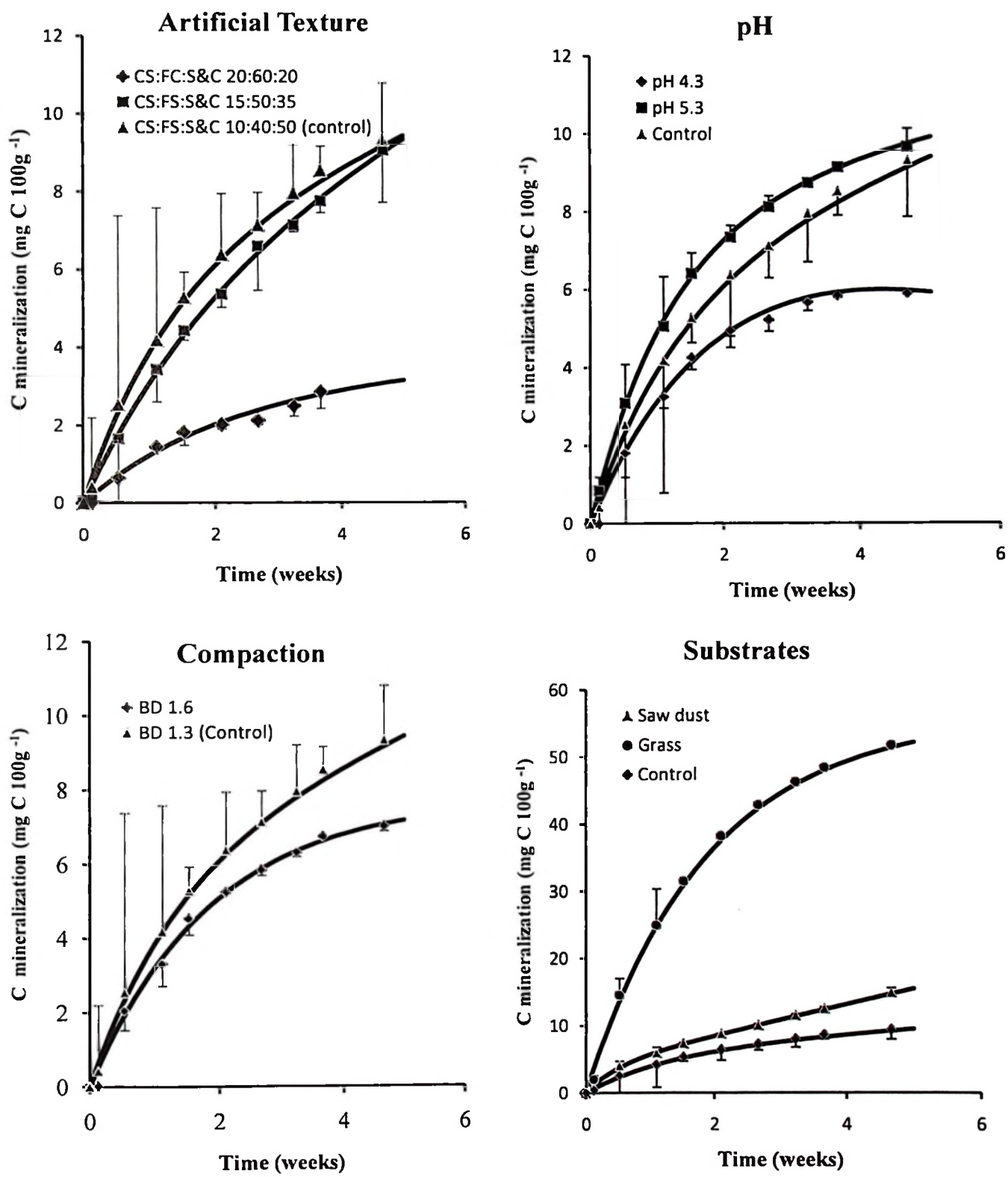


Figure 12 Cumulative C mineralization expressed in $\text{mg C } 100\text{g}^{-1}$ soil for the compaction, pH, Organic matter additions and texture treatments after five weeks incubation of soil (Error bars represent standard deviations of 3 replicates)

Table 2 Estimated parameters of the fitted parallel first and zero order kinetic model $C(t) = CA_f (1 - EXP(-k_f * t)) + k_s * t$ from the 1st experiment

Treatments	CA (mg C 100 g ⁻¹)	k _f (week ⁻¹)	k _s (mg C 100 g ⁻¹ week ⁻¹)	R ²	C mineralized in 5 weeks (mg C 100 g ⁻¹)	C mineralized in 5 weeks (% of OC present)
Control	6.1	0.731	0.7	0.99	85.723	1.9
15:50:35	5.9	0.488	0.7	0.99	76.621	2.4
20:60:20	2.9	0.494	0.1	0.98	31.547	1.7
pH 4.3	6.3	0.686	0.0	0.99	59.430	1.3
pH 5.3	8.1	0.801	0.4	1.00	99.524	2.2
BD 1.6	7.6	0.543	0.0	0.99	65.380	1.5
SAW DUST	3.9	1.924	2.3	1.00	153.997	2.8
GRASS	56.1	0.535	0.0	1.00	519.747	9.5

different treatment groups. The grass treatments had the highest CA_f (fast mineralizable C expressed in mg C 100 g⁻¹) value (56.1 mg C 100 g⁻¹), and the lowest value was observed in soil with artificial texture of CS: FS:S&C 20:60:20 (2.9 mg C 100 g⁻¹). The general trend with a decreasing order was as follows: grass, pH 5.3, BD 1.6, pH 4.3, Control, 15:50:35, sawdust, and 20:60:20.

The C first and zero order mineralization rates (k_f and k_s) differed among treatments, where for the case of mineralization rate constant of fast degradable C pool (k_f), values ranged from 0.488-1.924 week⁻¹ (Table 2). The highest value for k_f was observed for the saw dust treatment and the lowest value in CS: FS: S&C 15:50:35.

The mineralization rate constants of the slow C pool (k_s) were low ranging from 0 – 2.3 mg C 100 g⁻¹ week⁻¹ (Table 2). The highest value was found in Saw dust treatment and the lowest value from pH 4.3, BD 1.6 and grass treatments. These last three treatments had a k_s value of 0 mg C 100g⁻¹week⁻¹ implying that decomposition of the rapidly mineralizing pool extended beyond the here investigated incubation time. Considering the relative mineralization of 5

weeks, it became that the grass material was relatively better degradable than the sawdust as the relative proportional of C mineralized was more than three times as high.

Fig.13 shows the total C mineralization after five weeks incubation period from all the treatments. A statistically significant higher C mineralization was observed in the Grass treatment, but evidently it should be taken into account that mineralization for this treatment was the sum of C mineralization from SOM and the added OM. Regarding all other treatments, there were no significance differences in C mineralization according to Duncan's post hoc test.

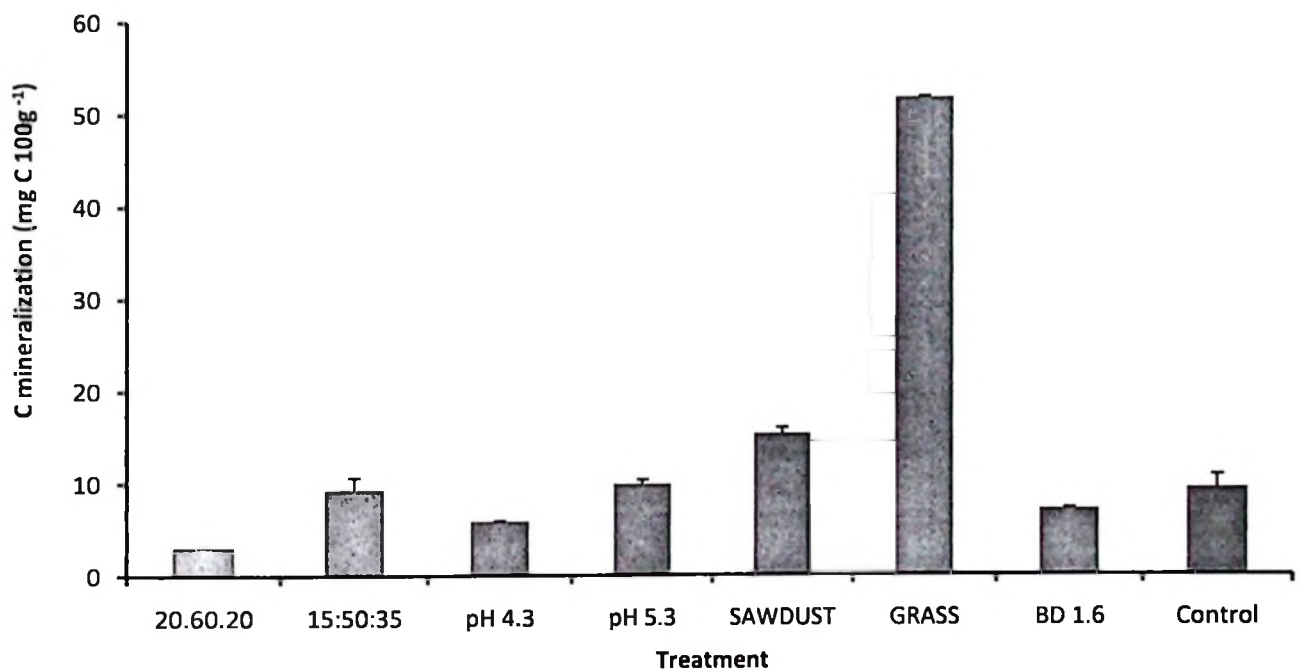


Figure 13 Cumulative C mineralization from the soil after 5 weeks incubation period from all treatments

4.1.2.2 Soil Microbial Biomass Carbon

The soil microbial biomass carbon (MBC) was determined for all treatments after incubating the soil samples for five weeks (Fig 14). Statistical analysis according to ANOVA with Duncan Post Hoc test showed the highest MBC in grass treatment and the lowest MBC in artificially changed texture with CS: FS: S&C mixture of 20:60:20. No significant differences in MBC with the control were observed in the soil samples compacted to a BD of 1.6 g cm⁻³ nor in the soil samples amended with saw dust. MBC was found to decrease significantly

after acidifying the soil, with a decreasing order as the pH decreases from 6.3 (control) to values 5.3 and 4.3, and after artificially changing the soil texture as amount of sand fractions increased from a CS:FS:S&C ratio 10:40:50 (control) to 15:50:35 and 20:60:20.

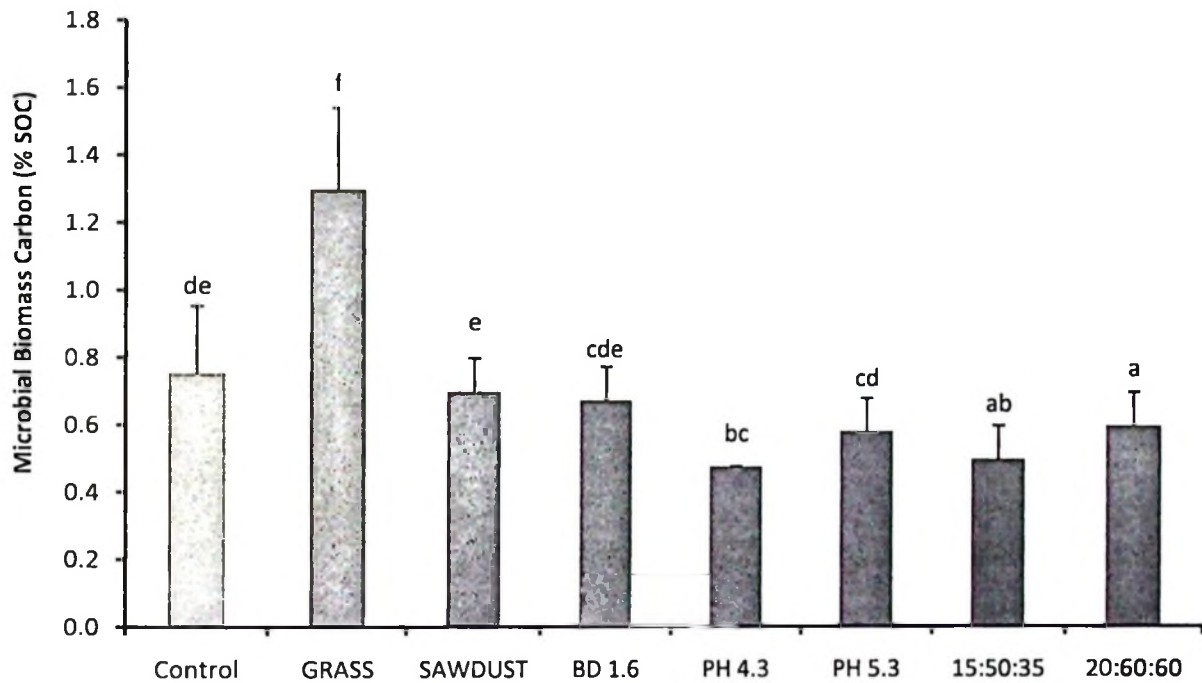


Figure 14 Microbial Biomass Carbon after 5 weeks soil incubation period from all treatment (Treatments presented by the same letter are not statistically different)

4.1.3 Microbial community composition (Bacteria/Fungi ratio) from PLFA extractions

The ratio of the two major decomposer groups (Bacteria and Fungi) was determined after incubation of soil samples for five weeks followed by PLFA extractions from all treatments. Fig 15 and 16 show the average bacteria/fungi ratio (B/F) of three replicates per treatment. All samples were processed in two batches of 24 samples (Table 3). Due to large differences between batches, the grass and sawdust and control treatments could only be compared among each other on the one hand (Fig 15) and the acidification, compaction and artificial texture and control treatments on the other (Fig 16). Statistical analysis by One way ANOVA followed by Duncan's post hoc test showed no significant difference in the B/F ratio from grass and sawdust treatments compared to the control soil. Soil compaction to BD 1.6 g cm⁻³ and pH 5.3 treatments were found to have no significant effect on the B/F ratio of these two decomposer groups. A very large variability among the replicates appears to have precluded

detecting significant differences between the substrate addition treatments. Absolutely, a clear lower B/F ratio was however, measured in the sawdust treatment when compared to the grass treatments. Significant lower B/F ratios were observed as the soil pH decreased to value of 4.3 where B/F ratio decreased from 28.24 at pH 6.3 (control) to 24.75 at pH 4.3.

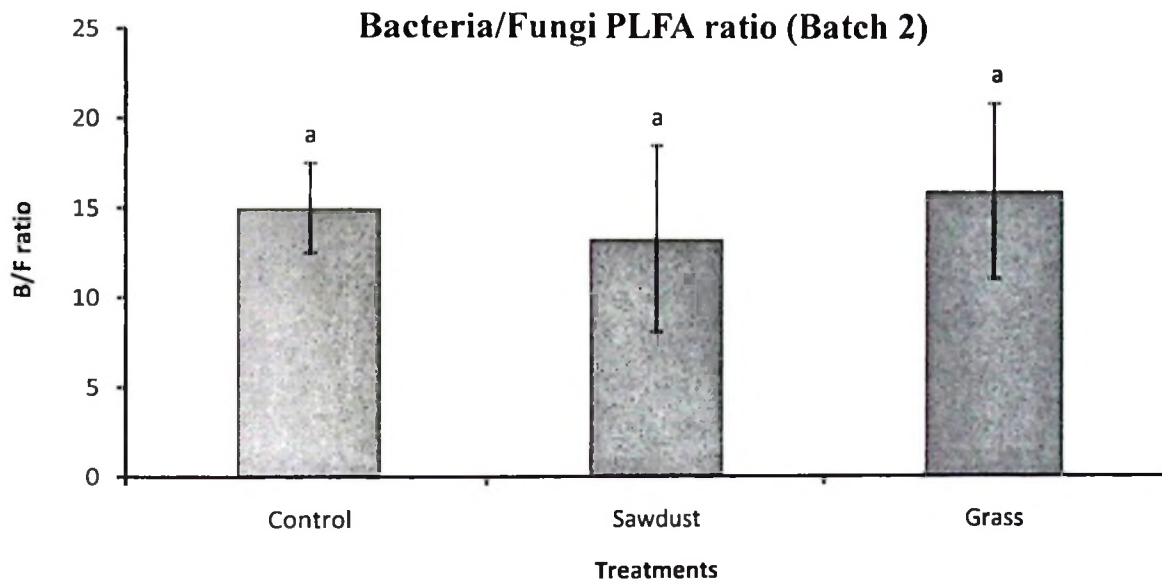


Figure 15 Bacteria to Fungi PLFA marker ratio from five weeks incubated soil samples where Grass and Sawdust organic materials were applied (Treatments presented by the same letter are not statistically different)

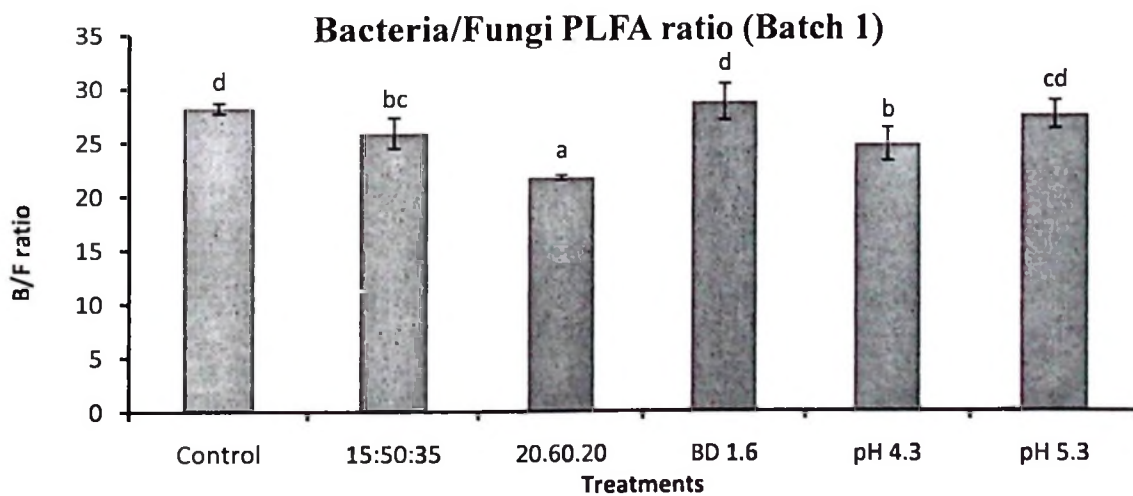


Figure 16 Bacteria to Fungi PLFA marker ratio from artificial manipulated texture, Compaction and pH treatments after five weeks incubation of soil samples (Treatments presented by the same letter are not statistically different)

An artificial change in soil texture towards coarse texture also led to a significant decrease of the B/F ratio with values 28.24, 25.91 and 21.74 for CS: FS: S&C of 10:40:50 (control), 15:50:35 and 20:60:20, respectively.

Table 3 Parameters of the microbial composition (Bacteria/Fungi ratio) from PLFA extractions

Batch	Treatments	Total nmol/g	Gram(+) bacteria (i and a)	gram(-) bacteria (cyC17+cyC19)	Fungi (C18:2c9,12)	bacteria/fungi
1	Control	19,78	3,77	1,15	0,19	28,24
	15:50:35	18,41	3,33	0,93	0,18	25,91
	20.60.20	12,87	2,27	0,55	0,15	21,74
	BD 1.6	20,98	4,04	1,23	0,20	28,73
	pH 4.3	16,95	3,41	0,91	0,19	24,75
	pH 5.3	18,50	3,69	1,04	0,19	27,48
2	Control	72,95	14,49	4,19	1,35	15,03
	Sawdust	70,03	14,26	3,97	1,59	13,31
	Grass	68,75	13,75	4,40	1,16	15,98

4.2 Second Incubation experiment

4.2.1 Carbon mineralization

In this experiment, C mineralization was determined by CO₂ headspace analysis during the incubation of small soil columns (with dimensions 1cm diameter*1cm height). Fig 17 and 18 show graphs from the cumulative measured C mineralization as a function of incubation time and the fitted parallel first and zero order mineralization kinetics model for the different treatments including compaction, substrates application (sawdust and grass) and artificial change in soil texture, CS: FS: S&C of 20:60:20. In general the R² values for all the

treatments were close to 1, implying that the selected model could describe the mineralization process appropriately. In all control treatments (BD 1.3, 1.6 g cm⁻³, CS: FS: S&C 20:60:20) and treatment with BD 1.6 with sawdust substrates, the first term of the model (First order mineralization kinetics) found to be almost zero. The evolution of C mineralization from these treatments therefore was nearly linear with time as also visible from the model parameters in Table 4, where the mineralization rate constant of the fast mineralizing OM pool (k_f) found to be near 0.

The parameters of the first plus zero order kinetic model varied between different treatment groups. The soil with artificial change of texture to CS:FS:S&C 20:60:20 with grass addition had the highest CA_f (fast mineralizable C as expressed in mg C kg⁻¹) value (387.26 mg C kg⁻¹), and the lowest value was observed in both compacted and non compacted soil with no OM addition (47.01 and 41.04 mg C kg⁻¹ respectively) (Table 4).

Table 4 Estimated parameters of the fitted parallel first and zero order kinetic model $C(t) = CA_f (1 - EXP(-k_f * t)) + k_s * t$ from the 2nd incubation experiment

Treatments	CA (mg C kg ⁻¹)	kf (week ⁻¹)	ks (mg C kg ⁻¹ week ⁻¹)	R ²	C mineralized in 5weeks (mg C kg ⁻¹)	Relative C mineralized in 5weeks (% of C present)
BD 1.3 Grass	112.66	1.720	35.84	0.99	295.40	5.2
BD 1.6 Grass	154.87	0.104	14.15	0.99	135.91	2.4
BD 1.3 Sawdust	272.84	0.184	6.59	0.99	199.70	3.5
BD 1.6 Sawdust	108.30	0.000	22.39	0.98	114.71	2.0
BD 1.3 Control	41.04	0.000	24.55	0.98	125.23	2.8
BD 1.6 Control	47.01	0.000	19.92	0.99	101.63	2.3
20:60:20 Grass	387.26	0.118	14.89	0.97	251.05	8.2
20:60:20 Sawdust	104.12	0.107	12.64	0.98	108.27	3.5
20:60:20 Control	65.25	0.000	17.38	0.98	88.66	4.6

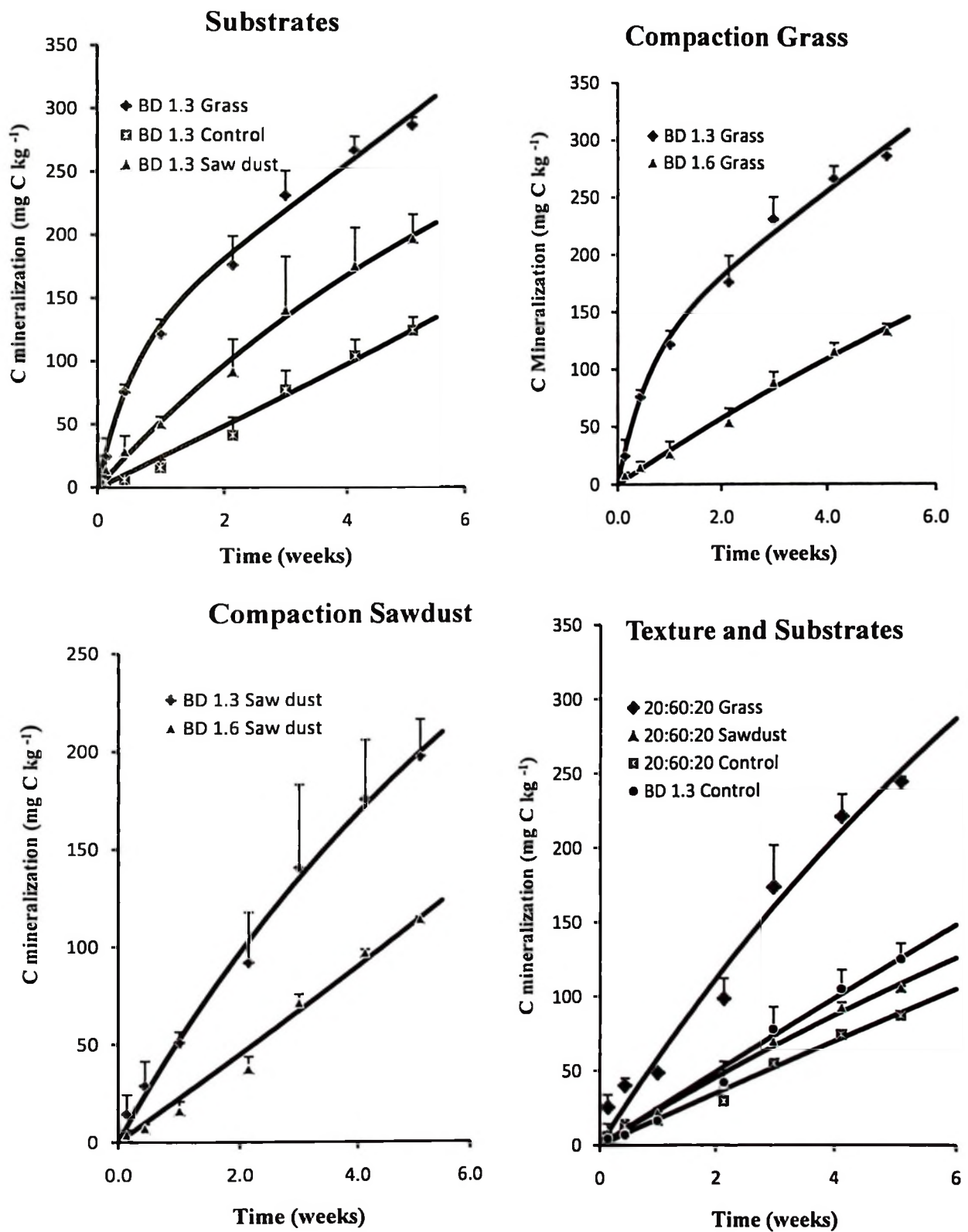


Figure 17 Cumulative C mineralization expressed in mg C kg⁻¹ soil for substrates addition, compaction, and texture treatments after five weeks incubation of soil (Error bars represent standard deviations of 3 replicates)

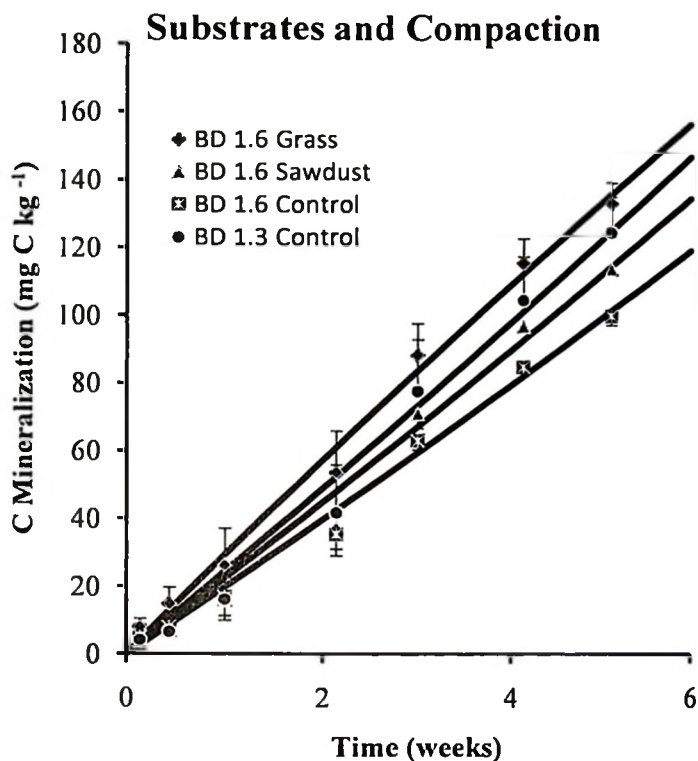


Figure 18 Cumulative C mineralization expressed in mg C kg⁻¹ soil for substrates addition and compaction treatments after five weeks incubation of soil (Error bars represent standard deviations of 3 replicates)

The C first and zero order mineralization rates (k_f and k_s) also differed among treatments. The mineralization rate constant of the fast degradable C pool (k_f), ranged from 0 - 1.72 week⁻¹ (Table 4). The highest value of k_f was observed with the non compacted soil (bulk density 1.3 g cm⁻¹ with grass addition and the lowest value was found in the Control soil (non compacted and no OM addition) and in compacted soil (bulk density 1.6 g cm⁻¹) where sawdust was added. The mineralization rate constants of the slow C pool (k_s) ranged from 6.59 – 35.84 mg C kg⁻¹ week⁻¹ (Table 4). The highest value was found in non compacted soil (bulk density 1.3 g cm⁻¹) with grass application and the lowest value in non compacted soil (bulk density 1.3 g cm⁻¹) with sawdust application.

Fig 19 shows the total C mineralization after five weeks incubation period from all the treatments. Statistical results from one way ANOVA followed by Duncan post hoc test after five weeks incubation showed higher C mineralization in non compacted soil (bulk density 1.3 g cm⁻¹) than in compacted soil (bulk density 1.6 g cm⁻³) after grass application. An almost similar trend was observed after application of Sawdust material for both compacted and non

compacted soils (Fig 19). Soil compaction at bulk density 1.6 g cm^{-3} without any application of substrate had no effect on C mineralization. Comparing the C mineralization from the two substrates in compacted soil, no significant differences were observed. This implies a strong effect of compaction on C mineralization overshadowing the difference in substrate type. Changing the soil texture artificially without any application of substrates had no effect on C mineralization. Changing soil texture from a CS: FS: S&C ratio of 10:40:50 to 20:60:20 with an application of either grass or sawdust materials had no significant effect on C mineralization but a consistently lower C mineralization was measured in the soils with artificial change in texture. But comparing the two substrates in both soil texture (CS: FS: S&C ratio of 10:40:50 to 20:60:20), C mineralization was statistically higher in soil with grass treatment than sawdust treatment. This indicates a strong effect of substrate type in C mineralization.

The relative OC mineralization within the 5-weeks period (Table 4) from SOM and the added OM materials for the non compacted soils (bulk density 1.3 g cm^{-1}) increased almost twice of what was observed in control when grass was applied (5.2 and 2.8 % of SOC respectively). For the compacted soil (bulk density 1.6 g cm^{-3}) with the same treatment, there was no increase in relative C mineralization since relative C mineralization was almost similar to control (Table 4). Almost similar trend was observed where sawdust was applied, and this implies the strong effect of compaction in C mineralization for both substrates. Comparing the two substrates, a higher relative C mineralization was found in grass materials than in Sawdust materials implying higher degradability of grass than sawdust. Changing soil texture to CS: FS: S&C ratio of 20:60:20 for grass treatment resulted to almost double relative carbon mineralization compared from what was found in control (8.2 and 4.6 % SOC) respectively (Table 4). An opposing result was found in sawdust treatment, where relative C mineralization decreased from 4.6-3.5 % of the OC equivalent to 24% decrease.

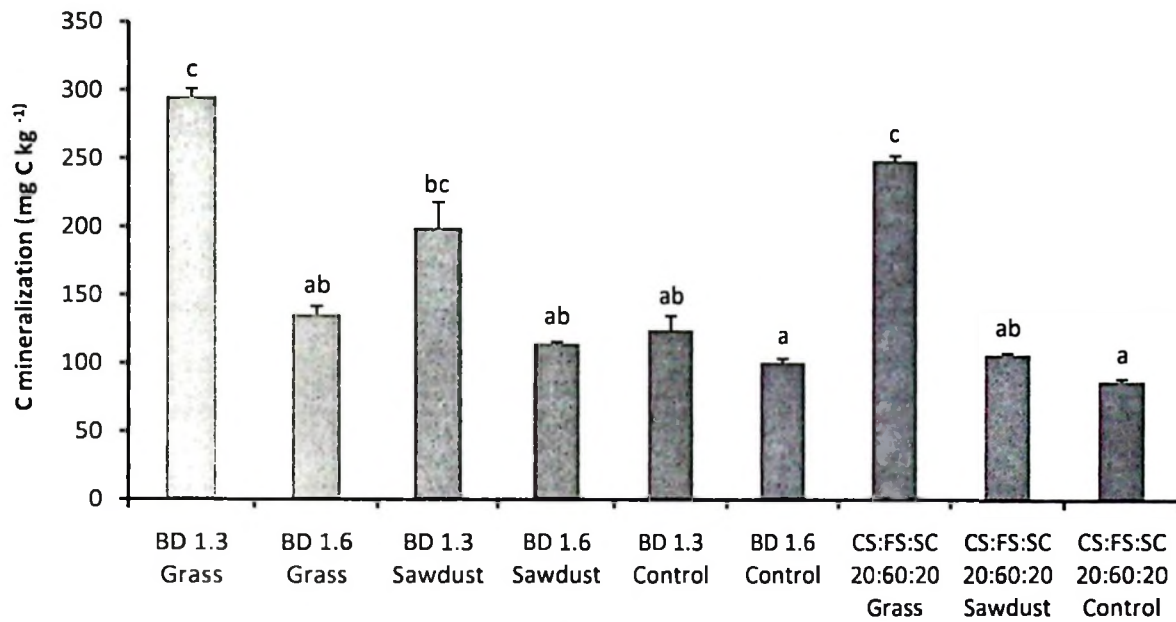


Figure 19 Cumulative C mineralization from the soil after 5 weeks incubation period from all treatments. (Treatments presented by the same letter are not statistically different)

Fig 20 shows the net effect of compaction and artificial change of soil texture treatments on C mineralization after addition of both substrates (grass and sawdust). Both compaction and artificially changed texture showed a clear net effect on C mineralization from sawdust material. A negative net effect on C mineralization from the grass applied soil was observed only with the compaction treatment.

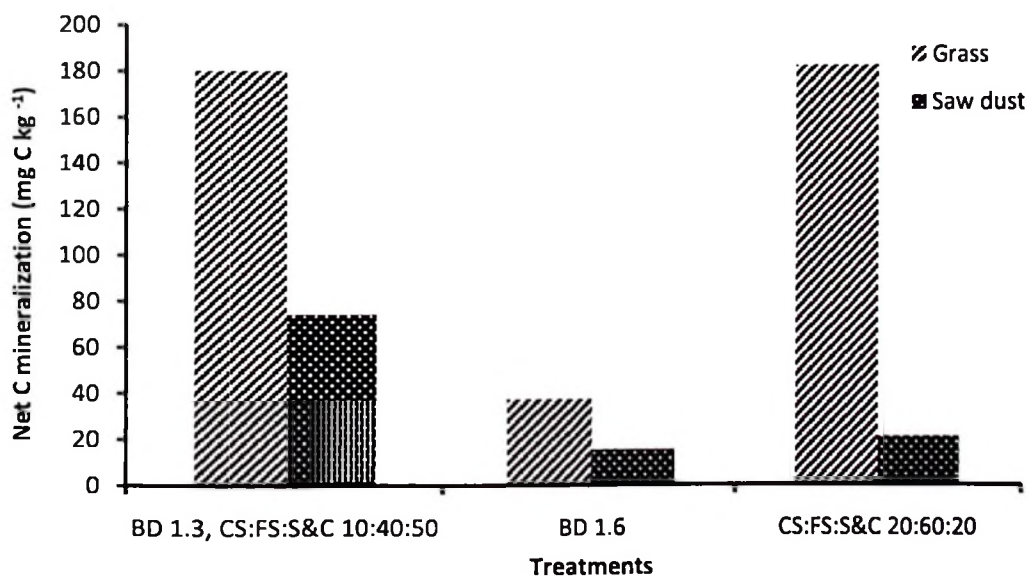


Figure 20 Net effect on C mineralization from the added substrates (grass and sawdust materials)

4.3 Image processing and calculation of the total soil pore volume (Porosity) and % pore volume from CT scans

Images representing the result of the 5 major image processing steps of the CT scanned soil columns before determination of total soil pore volume and % pore volume of different pore classes are presented in Fig 21. Fig 22 shows the total pore volume from all treatments derived from the CT scans. Total porosities calculated after image processing from CT scans were higher in non compacted soil (bulk density 1.3 g cm^{-3}) than in compacted soil (bulk density 1.6 g cm^{-3}) after grass application. A similar trend was observed after application of sawdust material in both compacted and non compacted soil. Application of grass material found to increase soil porosity significantly in both compacted and non compacted soils while no significant differences in soil porosity were observed after application of sawdust.

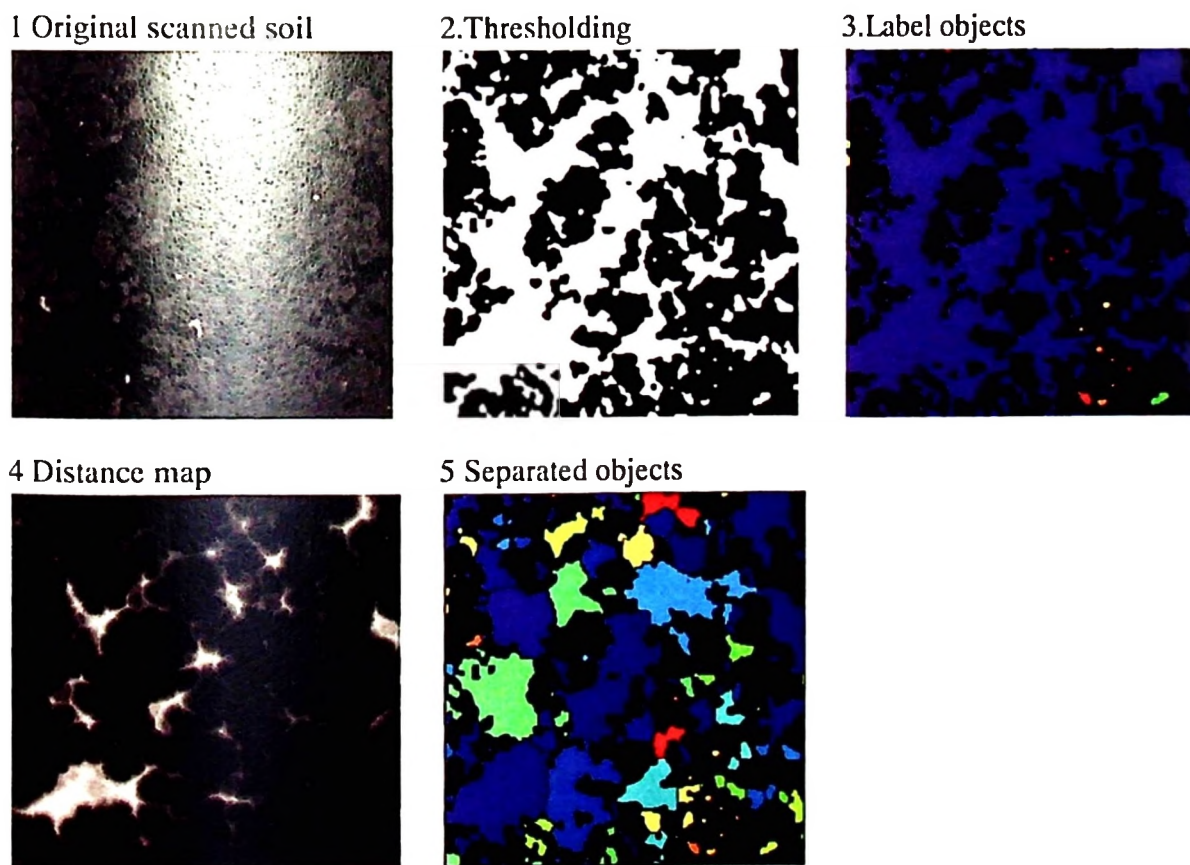


Figure 21 Images acquired during Image processing steps of the CT scanned soil column 2 in the determination of total soil pore volume and % pore volume of different pore classes. White in step 1, 2 and 4 represents pores and black the soil matrix

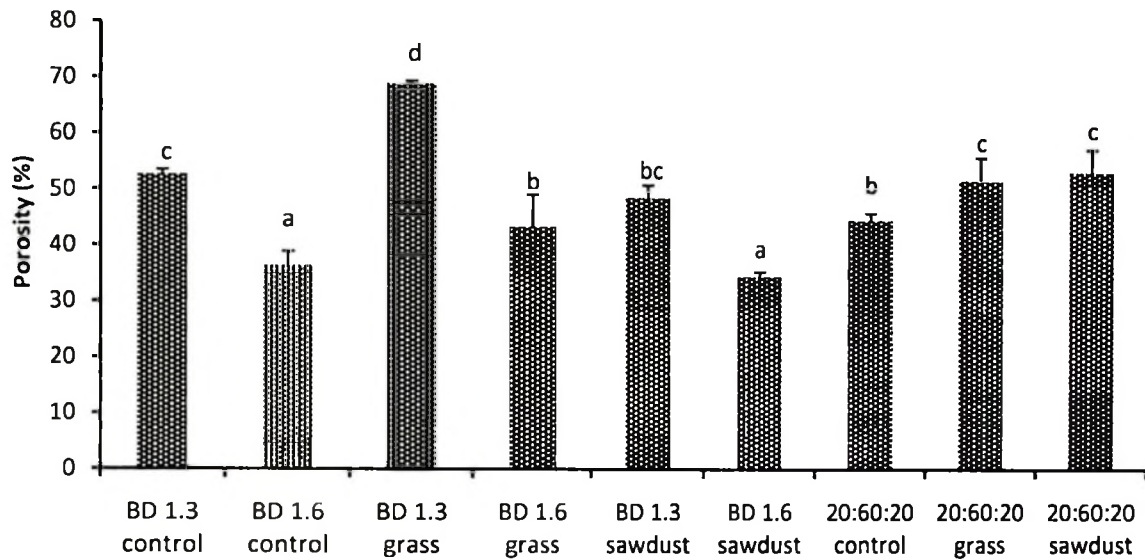


Figure 22 Total pore volume (Porosity) for all treatments derived from CT scans

Artificial change of soil texture from a CS: FS: S&C ratio of 10:40:50 (BD 1.3 control) to 20:60:20 without application of substrates decreased the porosity significantly while contrasting results were observed after application of substrates (grass or sawdust materials) in this treatment. However, the increase in soil porosity as influenced by the two added substrates was not statistically different from the BD 1.3g cm⁻³ - control.

Fig 23 shows the percentage pore volume of all pore classes determined after image processing of the soil scans. Compaction significantly reduced the % volume of >800 µm class in all soils and of the 410-600 µm class in the grass amended soils. When sawdust was added, both compacted soil (bulk density 1.6 g cm⁻³) and the soil with artificially changed texture CS: FS: S&C ratio of 20:60:20 showed a statistically higher % volume of pore class 10-200 µm than the control soil. Applications of either grass or sawdust in soil with artificially changed texture at a CS: FS: S&C ratio of 20:60:20 also resulted into statistically higher % volume of pore class >800 µm. However, similar result was found after application of grass materials in non compacted soil (bulk density 1.3 g cm⁻³).

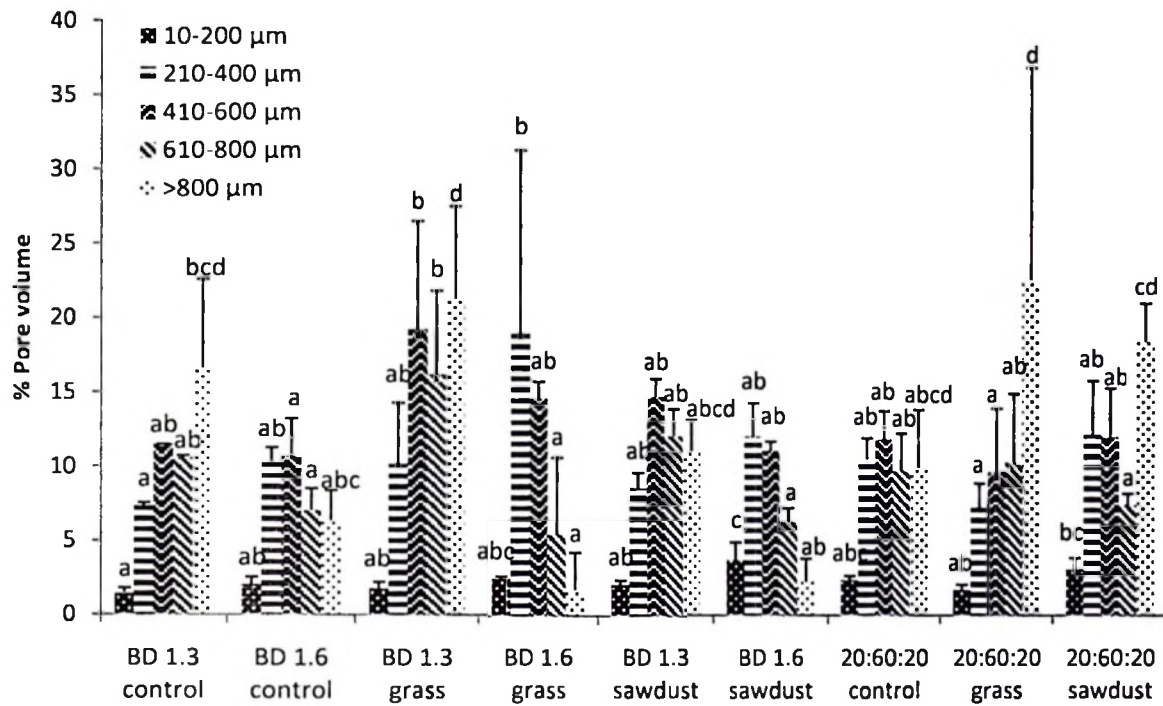


Figure 23 Percentage pore volume of all pore classes from all treatments, determined from CT scans (Treatments presented by the same letter are not statistically significant ($P = 0.05$))

Pearson's correlation analysis between the cumulative C mineralization after 5 weeks (mg C kg^{-1}) and the pore size distribution as derived from the X-ray scans was performed. Correlation coefficients were calculated between the C mineralization and the volume % of 5 different pore size classes, namely 10-200, 210-400, 410-600, 610-800 and $>800\mu\text{m}$ equivalent sphere diameter. First correlation analysis was done for the unamended soils to investigate the relation between C-mineralization from the native SOM and the soil pore size distribution (Fig 24). In the unamended soil, negative correlations between the cumulative C mineralization and total soil porosity was observed. C mineralization found to correlate positively with % pore volume of pore classes 210-400 μm and class 410-600 μm , and negatively with % pore volume of pore classes 10-200, 600-800 and $>800\mu\text{m}$. However none of these correlations were statistically significant.

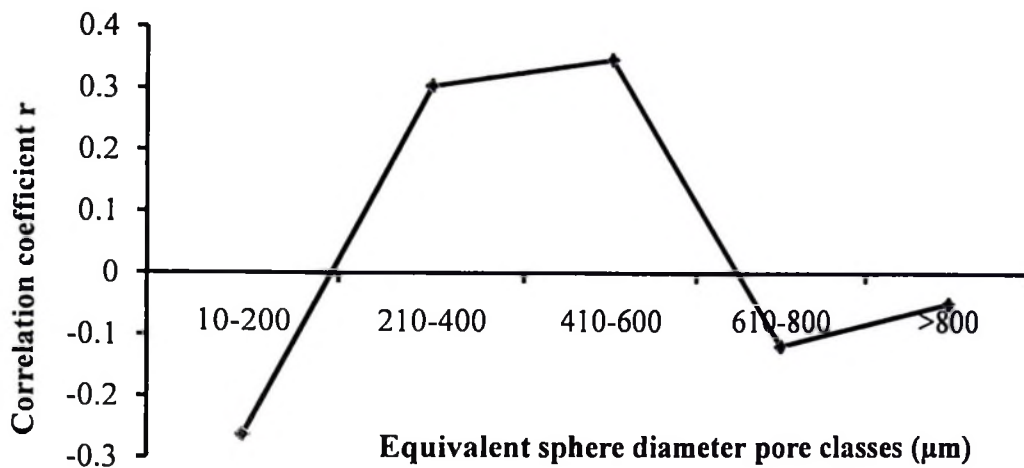


Figure 24 Correlation between cumulative C mineralization after 5 weeks incubation and the different pore-class volumes plotted against the pore equivalent sphere diameter derived from the X-ray CT volumes. (In unamended soil)

Second, for the grass amended soil columns (6 in total) (fig 25), a positive correlation existed between cumulative C mineralization after 5 weeks and total soil porosity but this correlation was not statistically significant. A significant negative correlation ($P= 0.01$) was found between C mineralization and % pore volume of the equivalent sphere diameter pore class 10-200 μm while a significant positive correlation ($P= 0.05$) were found in pore class 610-800 μm (Fig 26). There was a clear trend from negative towards positive correlation coefficients with increasing equivalent sphere diameter pore size.

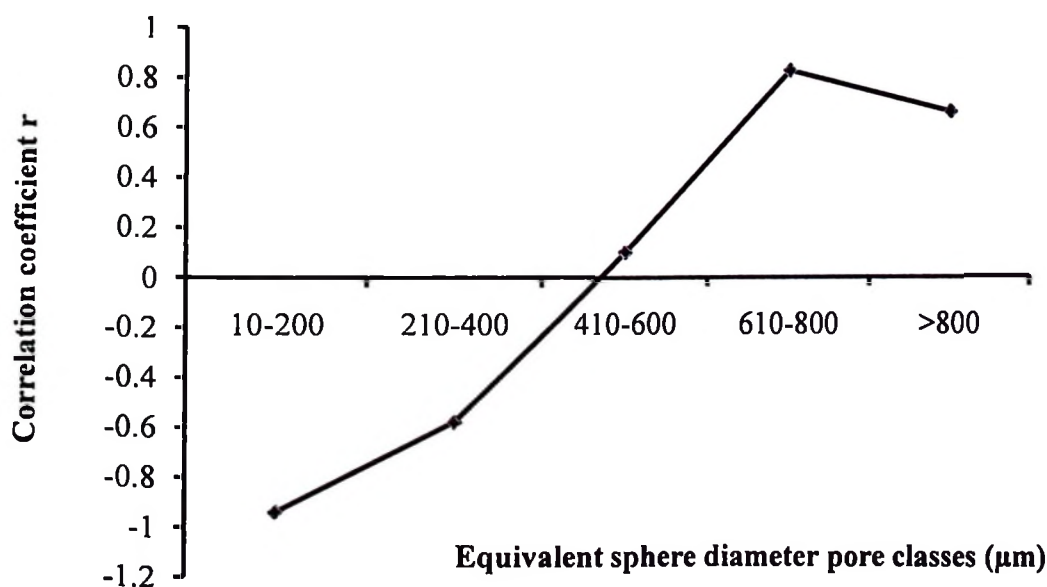


Figure 25 Correlation between cumulative C mineralization after 5 weeks incubation and the different pore-class volumes plotted against the pore equivalent sphere diameter derived from the X-ray CT volumes. (In soil amended with grass materials)

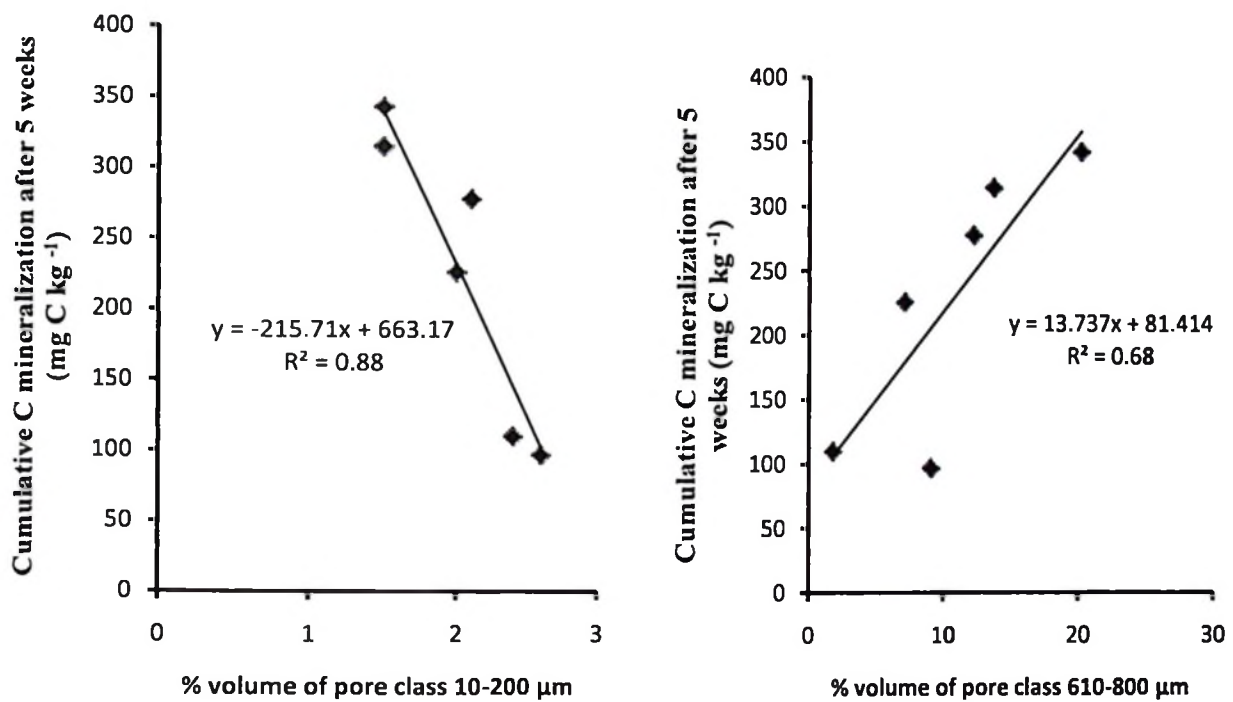


Figure 26 Linear relationship between cumulative C mineralization after 5 weeks and the % pore volume of 10-200 μm and 610-800 μm equivalent sphere diameter sized pore classes in the grass amended soil.

In the sawdust amended soils, there was significant positive correlation between cumulative C mineralization after 5 weeks and % pore volume of equivalent sphere diameter pore class 610-800 μm ($P = 0.05$) but not with the >800 μm class in contrast to grass amended soils (Fig 27). Correlations from all other pore classes and for total soil porosity were not statistically significant. Analogous to the grass amended soil columns, there was a trend towards positive correlation coefficients with increasing equivalent sphere diameter, except for the >800 μm class pores for which no correlation with C mineralization at all was found.

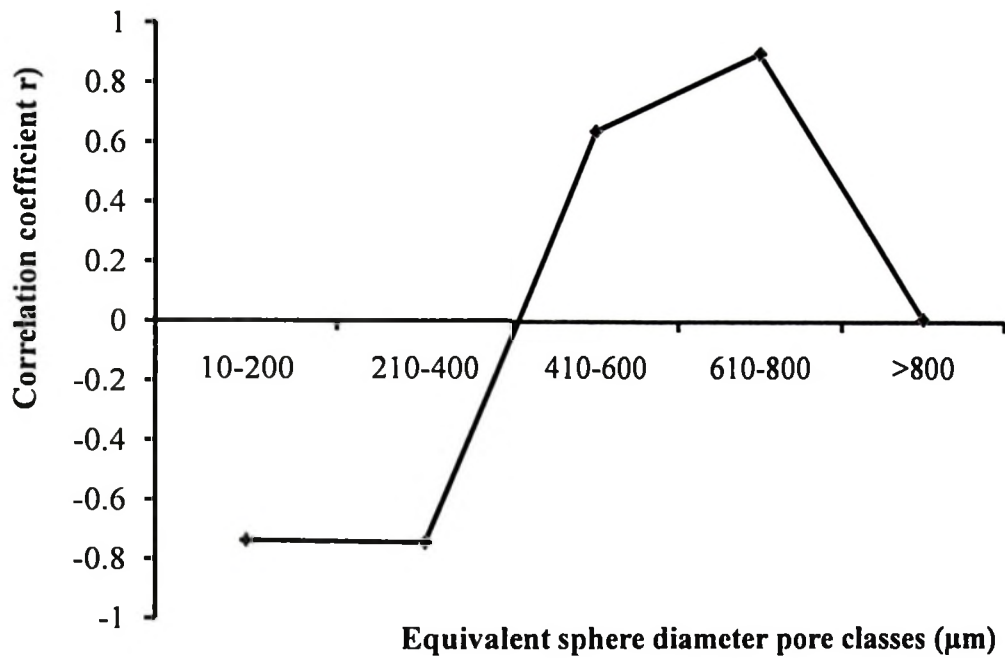


Figure 27 Correlation between cumulative C mineralization after 5 weeks incubation and the different pore-class volumes plotted against the pore equivalent sphere diameter derived from the X-ray CT volumes. (In soil amended with sawdust materials)

A final correlation analysis was carried out between the pore size distribution and net cumulative C mineralization from the added substrates after 5 weeks (mg C kg^{-1}) calculated by subtracting the C mineralization of the unamended from the amended soils (Fig 28). There was significant positive correlation between C mineralization and total soil porosity ($P= 0.05$) as well as with the volume % of pore class 610-800 μm . A very significant negative correlation ($P= 0.01$) was found between C mineralization and % pore volume in the pore class 10-200 μm .

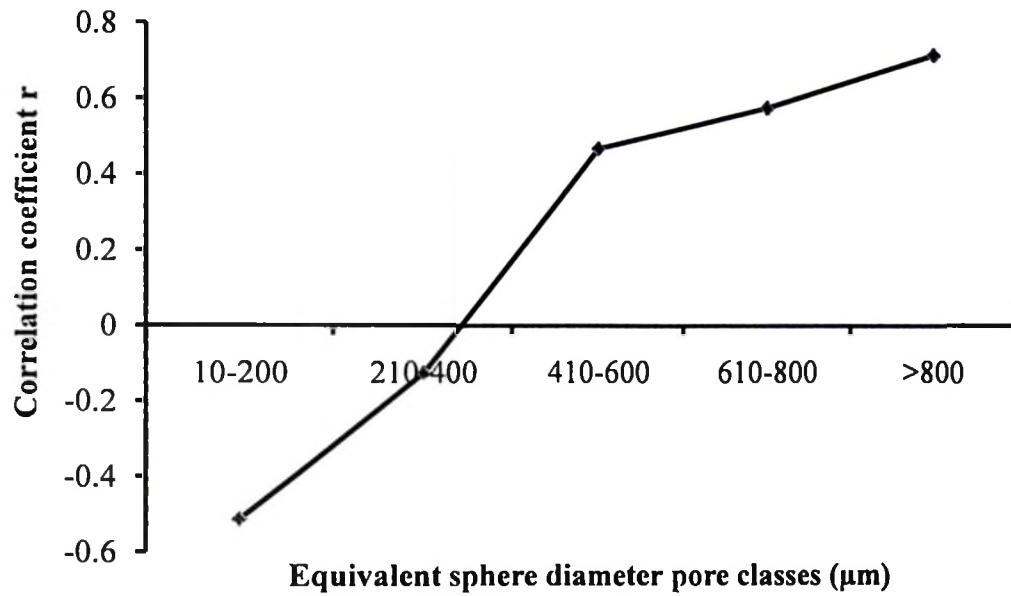


Figure 28 Correlation between net C mineralization (i.e. C mineralization of amended soil minus C mineralization of the corresponding unamended soil columns) after 5 weeks incubation and the different pore-class volumes plotted against the pore equivalent sphere diameter derived from the X-ray CT volumes. (For all soil treatments)

CHAPTER 5: DISCUSSION

5.1 Artificial soil manipulations and pF-Curve, aggregate size distribution and pore size distribution.

Artificial manipulations of the model sandy loam soil through compaction, OM application, soil acidification (lowering the soil pH) and artificial change of the soil texture affected the soil pore size distribution. From pF curve results (Fig 9 and 10), the dominance of small and intermediate sized pores (pores with neck diameter $<30\ \mu\text{m}$) was observed in all treatments.

The lower water content observed at every pressure in soil samples in which the soil texture was changed artificially from a CS: FS: S&C ratio of 10:40:50 to 15:50:35 and 20:60:20 indicate lower soil porosity, because of lower silt and clay content. Increasing sand fractions in the soil particle size distributions increased the bulk density on one hand and reduced the soil total pore volume (porosity) on the other. Low porosity in these treatments can also probably be explained by a lower amount of OM (OC 0.303 and 0.191% for CS: FS: S&C ratio 15:50:35 and 20:60:20 respectively), since presence of SOM improves soil structure through its influence on soil particles aggregation. The main effect of artificial change of soil texture in pore distribution was a lower volume of pores with pore neck diameter from 0.2-15 μm (micropores), and this may be explained by a lower proportion of silt and clay in the more sand textured soils.

The comparatively low volume % of macropores (pores with pore neck diameter from 30-300 μm) after compacting the soil at bulk density $1.6\ \text{g cm}^{-3}$ points to the loss of macropores. A similar result was observed by Breland et al (1996), who reported reduction of total soil pore volume (porosity) and changes of the pore size distribution after soil compaction, towards a higher percentage of small pores. In that study, using a sandy loam soil at bulk density 1.1 and $1.4\ \text{g cm}^{-3}$, soil compaction was also found to reduce the volume of pores with neck diameter $> 30\ \mu\text{m}$, i.e. pores available to nematodes. The results are also in agreement from what was observed by Shestak and Busse (2005) who observed a significant effect of compaction on macroporosity after 67 days laboratory incubation of a sandy loam soil.

The soil porosity of the grass application treatment was slightly higher than in control treatment. Probably, this can be attributed to higher microbial activities in the grass applied soil due to the presence of an easily degradable food source (low C:N ratio of 24) which stimulates microbial activities. Higher microbial activities enhance soil aggregation resulting

to improved soil structure. A similar observation may be made for the pH 5.3 treatment but an immediate explanation to improved soil structure is not readily found.

The wet sieving method (Fig 8) was unable to deliver any usable information on aggregates and pore size distribution because of very weak soil aggregate stability in all soil treatments. This probably can be explained by the sandy texture and very low OC content (0.191-0.448%) in the reconstructed soils.

In summary, artificial change of soil texture mainly affects small pore sizes and also large pores, while soil compaction at bulk density 1.6 g cm^{-1} reduces only pores $> 30 \text{ }\mu\text{m}$. Therefore this points to a scope for using these treatments to manipulate the pore structure in incubation studies, which was the main point of interest in this first experiment.

5.2 Artificial soil manipulations and C mineralization, microbial biomass C and Bacteria/Fungi ratio

5.2.1 Artificial soil manipulations and microbial biomass C

Apart from total amounts of OC, the MBC, being a living fraction of OM, has been suggested to be a useful and sensitive measure of a change in OM status (Powlson et al, 1987). Changes in the MBC can thus provide an early indication of longer-term trends in the total OC of soils (Powlson et al, 1987). A significant decrease in MBC observed after acidifying the soil from pH 6.3 (control) to pH values 5.3 and 4.3 can at least partly be related to the effect of soil acidity on microbial community distribution. Considering the major two decomposer groups which were determined (fungi and bacteria) probably mostly bacteria were affected by soil acidification. Rousk et al (2009) found a strong decrease in bacterial growth rate (80%) after pH drop from 7 to 4.5, with the lowest bacteria growth rate at pH 4, where the decrease reached 90%.

The lowest amount of MBC was found after artificially changing the soil texture as amount of sand fractions increased from CS: FS: S&C ratio 10:40:50 (control) to 20:60:20. Since the MBC normally comprises only 1-5% of the total SOC, this could evidently be attributed to the lower amount of SOC in this treatment (0.191%) compared to control soil (0.448%). The highest MBC observed in the grass treatment can be explained by the input of a food source for microbes in the grass material, which with C:N ratio (24) and being rich in carbohydrate, is easily degradable and favours bacterial decomposition. This result can be linked to the highest C mineralization, observed as well (see 5.2.3).

Artificial manipulation of the soil through compaction and addition of sawdust material had little or no effect on MBC. This might imply that most microorganisms are distributed in micropores (pores with pore neck diameter $< 30 \mu\text{m}$), pores which were not affected by compaction thus microbial activities were not impaired. Low degradability of sawdust due to its high lignin content and high C:N ratio can be a factor for relatively low MBC (ie. not increased as compared to the control) observed in this treatment.

5.2.2 Artificial soil manipulations and microbial community composition

(Bacteria/Fungi ratio)

Bacteria and fungi are the two major groups of the soil microbial community responsible for decomposition of SOM, and they comprise $> 90\%$ of the total soil microbial biomass (Six et al, 2006). A decrease in bacteria to fungi ratio (B/F ratio) at pH 4.3 can be explained by the effect of soil acidity on distribution of microbial decomposers where, the lowest bacteria population is expected. On other hand increase in fungi population is expected when increasing soil acidity because of an increase in fungal respiration. Blagodatskaya et al (1998) reported an increase in respiratory activities of fungi under acidic conditions while that of bacteria decreased. Rousk et al (2009) also observed an increase in fungal to bacterial growth ratio with decreasing soil pH from 8.3 to 4.5 and the maximum growth ratio was reported at pH 4.5.

The ratio of the two major decomposer groups (Bacteria and Fungi) as determined after PLFA extractions was found not to be affected by soil compaction at $\text{BD } 1.6 \text{ g cm}^{-3}$ as well as by soil acidification at pH 5.3. Although there was large variability among the replicates which precluded detecting significant differences of these treatments from control, linking this result to what was observed in C mineralization and MBC results (see 5.2.1 and 5.2.3), confirms no effect of these treatments on distribution of fungi and bacteria communities. Since soil compaction found to reduce macropores only (pores with pore neck diameter $>30 \mu\text{m}$), probably most habitable pores to these microorganisms were not affected. Eliot et al (1980) reported that, pores of neck size diameter from $0.8\text{-}6 \mu\text{m}$ are accessible mainly to bacteria and the maximum diameter of the pores most frequently colonized by bacteria in coarse textured soils was estimated to be $9 \mu\text{m}$ (Postma et al, 1989). Strong et al (2004) reported a positive correlation between fungal biochemical makers and the volume of pore neck size class $15\text{-}60 \mu\text{m}$. This supports the explanation why compaction had little effect on both fungi and bacteria.

The lowest B/F ratio was observed after artificial change of soil texture from a CS: FS: S&C ratio 10:40:50 (control) to 20:60:20 and can be related to the dominance of fungi in sandy soils. A fungal population dominates in sandy soils because sandy soil has higher proportional of pores accessible by fungi thus their hyphae can bridge considerably across air filled pores (Strong et al, 2004). Also the porosity < 15 μm was strongly reduced by these treatments which is almost exclusively accessible to bacteria. Therefore low B/F could be explained by the effect of reduction in micropores (pores assessible to bacteria) to the bacteria population. Although there was no significant difference in the B/F ratio from grass and sawdust treatments compared to the control soil, a clear lower B/F ratio was measured to the sawdust treatment when compared to the grass treatments. This implies the presence of more fungi in the sawdust treatment. Which is expectable because of the sawdust's very high C:N ratio (244) and being rich in lignin, which is only degradable by specific fungi (Knabner 2002). Based on biomass measurement of bacteria and fungi, Thiet et al (2006) also observed the stimulation of fungal contribution to decomposition after application of OM with high C:N ratio (Straw with C:N ratio 75). Besides other factors like intensive cultivation, increased grazing pressure, increased N fertilization, Thiet et al (2006) also reported the importance of substrate quality in altering B/F ratio. Low quality substrates (high C:N ratio) tend to attract more fungi while high quality substrates tend to favour bacteria (Bossuyt et al, 2001).

5.2.3 Artificial soil manipulations and C mineralization.

Artificial manipulations of soil had clear effects on C mineralization. Soil compaction at bulk density 1.6 g cm^{-3} and pH 4.3 decreased C mineralization for about 23.7 and 26.3%, compared to the bulk density 1.3 and pH 6.3 (controls) respectively, after five weeks of incubation. The decrease in C mineralization cannot be related to the effect of compaction on distribution of microbial decomposer community because no effects were found (5:2:2). Since compaction reduced pores with pore neck diameter $>30 \mu\text{m}$, possibly the distribution of larger soil organisms such as nematodes and fungi, which can be accommodated in these pores could have been affected. More likely through the effect of compaction on C mineralization could have been indirect through its impact on aeration and moisture distribution. De Neve et al (2000) using loam sand soil, found no significant influence of soil compaction in microbial processes such as C mineralization. In that study the effect of compaction in microbial processes like C mineralization from SOM (unamended soil) was noted to start after compacting the soil at bulk density 1.6 Mg m^{-3} . Since all the POM was removed from the soil samples, the effect of soil compaction in C mineralization could

alternatively related to an assumption suggested by De Neve et al (2000) that, concentration of native SOM in the more stabilized fine fraction of OM pools associated with small pores, can be a factor for its mineralization being less influenced by soil compaction.

Acidifying the soil to a pH 4.3, negatively affected the abundance of most bacteria as seen from PLFA data (5:2:2). A clear drop in C mineralization results from a logical lower bacteria activity in acidified soil.

Artificial change in soil texture showed a strong decrease in C mineralization especially for CS: FS: S&C ratio 20:60:20. Taking into account the small amount of initial SOC in this treatment (0.191%), the relative C mineralization, however, was not significantly different compared to the control soil. This implies that the C mineralization rate from the native SOM was not affected by changing texture artificially. The highest C mineralization, observed in grass treatment, logically could be attributed to the easily degradability of this grass material since its C:N ratio was low (24) compared to that of sawdust (244). Grass materials being rich in carbohydrates, bacteria are expected to dominate in this decomposition. In comparison to sawdust, which was added as another treatment, low C mineralization observed could be attributed to the nature of the material being rich in lignin, and with high C:N ratio (244) thus not easily degraded.

5.3 Relation between soil pore structure, and C mineralization (2nd experiment)

5.3.1 Artificial soil manipulations and total soil pore volume

Porosity and pore size distributions were obtained from image analysis from the X-ray CT scans of the small soil cores (Fig 22 and 23). In general shift in pore size distribution were in line with the pF data obtained in the first experiment. Again as in the first experiment, the higher total soil pore volume (soil porosity) observed in a non compacted soil (BD 1.3g cm⁻³) than in compacted soil (BD 1.6g cm⁻³) was also accompanied by changes in pore size distribution. Here the soil compaction was found to affect the larger pores (macropores) only which fits with pF curve data from the first experiment. The comparatively high volume % of pores with equivalent sphere diameter from 10-200 µm after compacting the soil at bulk density 1.6 g cm⁻³ points to an increase in pores of this class. Similar result was observed by Breland et al (1996) who reported changes of the pore size distribution after soil compaction,

towards a higher percentage of small pores. In that study using sandy loam soil at bulk density 1.1 and 1.4 g cm⁻³, soil compaction was also found to increase the volume of pores with neck diameter < 3µm. But in this study, CT was used, which allowed only observations of pores > 10µm equivalent sphere diameter due to resolution limit. An increase in soil porosity after addition of substrates in non compacted soils can be related to improvement of soil structure as influenced by higher microbial activities and by the physical action of the substrate itself after OM additions (50-200 µm sized OM particles). The higher soil porosity observed after application of grass materials than sawdust may be associated to degradability of these materials. Grass materials addition favoured microbial activity as seen from the C mineralization data, thus enhancing the soil structure. Total soil porosity was found to decrease significantly after artificial change of soil texture from CS: FS: S&C ratio of 10:40:50 to 20:60:20. This could be attributed to the effect of increasing sand fractions in the soil particle size distribution, which probably decreases soil microporosity. However, an increase in soil porosity with addition of either of the two substrates, even with artificially changed texture, indicates an improvement of soil structure as influenced by addition of OM. At the same time, here macroporosity was improved as seen from X-ray CT data while microporosity possibly reduced. The later could not be verified as such a combined soil treatment was not included in the first experiment and the spatial resolution of the X-ray data was too limited.

No or only little change in the % pore volume of pores 10-200 µm equivalent sphere diameter after artificial change in soil texture from CS: FS: S&C ratio of 10:40:50 to 20:60:20 and application of sawdust or grass materials was found. Comparing this result from pF result in first experiment where the micropores (pores with pore neck diameter 0.2-15µm) found to decrease significantly after artificial change of soil texture, the % pore volume of pores 10-200 µm equivalent sphere diameter even increased slightly in the sawdust added soil of CS: FS: S&C ratio 20:60:20. This result probably contradicts the outcome of the pF curve, in which it was found that microporosity was reduced by change in soil texture. This difference may possibly be explained by differences in methodologies: quantification of equivalent sphere size vs. pore neck diameter. Otherwise, this could be related to the improvement of soil structure after substrate (sawdust) addition which was, however, less pronounced as seen from the pF data. A significant increase in % volume of pores > 800 µm for artificially changed soil texture after addition of substrates can be related to the positive influence of

substrates. The higher proportion of macropores from pF results are comparable from what was observed here (higher % pore volume >800 μm equivalent sphere diameter).

5.3.2 Artificial soil manipulations and C mineralization

C mineralization from the second experiment was determined from a mixture of treatments where two contrasting substrates (grass and sawdust) were applied in non compacted soil (BD 1.3 mg cm^{-3}), compacted soil (BD 1.6 mg cm^{-3}) and soil with artificially changed texture (CS: FS: S&C of 20:60:20). Grass and sawdust applications resulted clearly in elevated C mineralization and by subtracting the C mineralization assessed from the unamended soil, net substrate mineralization could well be calculated. The application of grass in non compacted soil (BD 1.3 mg cm^{-3}) resulted into an almost double amount of C mineralized after five weeks in comparison to grass application in compacted soil (BD 1.6 mg cm^{-3}). An almost similar trend was observed for the sawdust treatment, and this demonstrates the strong effect of compaction, rather than substrate type, on C mineralization. Since the inhibiting effect of compaction was observed mainly when substrates were added and to a lesser extent without substrate application, there appears to be an interaction effect from both factors (compaction and substrate addition). The net negative effect of compaction on C mineralization was found to be larger with grass than with sawdust addition, also implying an additional interaction effect of substrate type.

Artificial change in soil texture from a CS: FS: S&C ratio of 10:40:50 to 20:60:20 without substrates application considerably reduced C mineralization when compared to unamended soil. When substrate was added, reductions of C mineralization were found for both grass and sawdust application but the inhibiting effect was much more pronounced for sawdust. Regarding the net C mineralization, no inhibition was found for grass with an artificial change in texture. Since the net negative effect on C mineralization was found only in artificially changed texture where sawdust was added, this points to a specific combination effect from the artificial change of soil texture and substrate type. This effect may be related to an indirect action on aeration, water availability or change in microbial community.

From the PLFA extraction result, the artificial change in soil texture from a CS: FS: S&C ratio of 10:40:50 to 15:50:35 and 20:60:20 was found to reduce the B/F ratio significantly, implying a relative increase in fungal biomass. An almost similar result was observed in sawdust amended soil, where also a considerable decrease in B/F ratio was observed. Consequently, the combination of both artificial change in soil texture and sawdust

application may be expected to promote fungi and fungal controlled OM decomposition. Moreover an artificial change in soil texture increased the proportion of larger pores, which enhances soil aeration on one hand and probably decreased the proportion of micropores (0.2-15 μm which are water storage pores) on the other hand. Baldock et al (2000) reported that changes in the pore size distribution towards a greater proportion of large pores, such as noted in progressing from clay to sand, are accompanied by higher rates of C mineralization. In that study, it was also noted that the fungal proliferation in pore class 15-60 μm resulted in fast decomposition of label-derived C in this pore region. Adu and Oades (1978) found that fungi were more efficient in mineralizing C in micropores than bacteria in a sandy loam soil amended with glucose or starch. Apparently, fungi benefit from presence of OM in smaller pores (<30 μm pore neck size) and these were strongly reduced with artificial change in texture as assessed from pF curve data in the first experiment. Linking these three scenarios (a relative shift towards a more fungal population, enhanced soil aeration and decrease in water storage pores), the net negative effect on C mineralization found after artificial change in texture with sawdust addition, possibly, might be attributed to limitations on C mineralization related to soil moisture availability. But the true nature of this effect is not clear yet.

5.3.3 Relation between soil pore size distribution and C mineralization

An attempt to further elucidate the role of differently sized pore classes in the C mineralization process was made by correlation analysis between the C mineralization data and the X-ray CT derived pore size distribution data. Results from Pearson's correlation analysis between the cumulative C mineralization after 5 weeks (mg C kg^{-1}) and pore size distribution from SOM (unamended soil) showed a positive correlation between cumulative C mineralization and pore sizes with an equivalent sphere diameter 200-400 μm and 410-610 μm , and negative correlation with equivalent sphere diameter pores class 10-200, 600-800 and >800 μm . Although these correlations were not statistically significant, probably, a link between native SOM C mineralization and the intermediate pore size volume can be attributed to favorable conditions for microbial activities resulting from a good balance between soil aeration and moisture availability in these intermediately sized pores. Baldock et al (2000) also reported that pore size classes which ensure adequate quantities of available water and oxygen optimize the processes of OM decomposition and mineralization. The

favourable conditions for microbial activities such as good soil aeration where good gas exchange (O_2 , CO_2) is expected.

Also, physical support to accommodate most microorganisms may be another factor for the possible enhanced microbial activities in larger pores (610-800 μm). Indeed, presence of nematodes and other fauna might enhance C mineralization. For example, Hassink et al (1994) estimated the faunal contribution to C mineralization to be 4-12% of the total C mineralization. Most (about 80%) of this faunal contribution to C mineralization would derive from an indirect positive effect on the growth rate of microbes. In contrast, low C mineralization in pore with equivalent sphere pore diameter 10-200 μm may be linked to possible low microbial activities in small pores. The tendency of these pores to stay saturated with water for a longer time could create anaerobic conditions affecting microbial activity. Also limitations to physically support larger microorganisms might have reduced the rate of microbial activities in small pores. Baldock et al (2000) reported the concept of exclusion, regarding the OM decomposition and the physical size of the decomposer organisms. Van der Linden et al (1989) showed that protozoa and nematodes are excluded from pores $<5 \mu\text{m}$ and $<30 \mu\text{m}$, respectively. Therefore, OM residing in pores smaller than these diameters will not be susceptible to decomposition or predation by these soil fauna.

incubation studies that employ combinations of compaction, change in texture, acidification and substrate addition. Following these first experiments, combinations of soil manipulations including grass or sawdust addition, soil compaction and artificial change in soil texture were used to study C mineralization in relation to soil pore structure. Very pronounced interaction effects on C mineralization between substrate type and the artificial changes in soil pore structure were found. First an interaction effect between soil compaction at BD 1.6 g cm^{-3} compared to BD 1.3 g cm^{-3} and substrate type was found, as the compaction induced reduction on C mineralization and this was more pronounced in grass than in sawdust amended soil. Similarly, there was an interaction effect between artificial change in texture and substrate type (sawdust), the combination of both strongly reduced the net substrates derived C mineralization after 5 weeks incubation period. The negative effect on C mineralization of these combinations of artificial operations derives from changes in soil pore structure and related effects on aeration, water distribution and microbial community.

Pore size distribution in the small soil cores were successfully quantified from 3D X-ray CT scans and computer image analysis. The volume of 5 arbitrary pore size classes was quantified on the basis of equivalent sphere diameter namely 10-200, 210-400, 410-600, 610-800 and $>800 \text{ }\mu\text{m}$. A positive correlation between cumulative C mineralization from native SOM and the % pore volume of the 200-600 μm equivalent sphere diameter class and negative correlations with % volume of pores 10-200 and $>600 \text{ }\mu\text{m}$ classes may suggest the dominance of C mineralization in intermediate pore size classes. This could be related to the presence of favourable microbial conditions such as good balance of air and water in such pores.

A very different outcome was observed with additions of C substrates such as grass and sawdust ($\pm 50\text{-}200 \text{ }\mu\text{m}$). In both grass and sawdust amended soil, a clear trend of the correlation coefficients from negative to positive with increasing pore size class suggest a positive influence of macroporosity on C mineralization. The positive correlation between cumulative C mineralization after 5 weeks and % volume of pores with equivalent sphere diameter 610-800 μm observed in both grass and sawdust amended soils, may derive from enhanced aeration and mineralization in more porous soil.

The inhibiting effect of soil compaction on C mineralization was likely related to the macropores reduction as observed from pF and X-ray CT data after soil compaction. Since there was no effect of compaction on micropores and on the distribution of the microbial

community, negative correlation between cumulative C mineralization from SOM after 5 weeks and the % volume of pores with equivalent sphere diameter 10-200 μm might not be linked to physical protection of SOM against biological degradation in small pores. Interestingly, the inhibitory effect of artificial change in soil texture on net substrate C mineralization was much more pronounced in the sawdust amended soil. Change towards a more sandy texture was confirmed to reduce microporosity (as opposed to compaction). Next for PLFA analysis sawdust addition was found to promote fungal biomass. Consequently the reduction of small pores (with pore necks $<30 \mu\text{m}$) must have strongly and negatively affected fungal decomposition of the added sawdust. A similar negative effect was not found for grass addition, which are probably bacteria dominated. The negative influence on C mineralization of change in soil texture and associated reduced microporosity may derive from a change in soil moisture distribution, but this is not clear.

In conclusion, interesting but complex interactions between soil pore structure and substrate type were demonstrated by these experiments. Pronounced negative or positive correlations between pore size class volumes and C mineralization could be established and this indicates their role in the OM decomposition process. At this point the nature of these effects of variations in a certain pore size class on C mineralization can only be hypothesized. Explanations could involve changes in aeration, moisture availability, shift in habitable pore space etc. and more in depth research is required to improve an insight on this matter. Controlled incubation studies in combination with X-ray CT and PLFA analysis would seem to be fit for this purpose.

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