SOIL MICROBIAL COMMUNITY, SOIL AGGREGATION AND CROPPING SYSTEM: STUDY OF THEIR RELATIONSHIP

Sabine Caniquitte
Department of Natural Resource Sciences,
McGill University, Montreal
May, 2008

A thesis submitted to the office of Graduate and Postdoctoral Studies in partial
fulfillment of the requirements for the degree of Doctor of Philosophy

Abstract

The effect of six cropping systems on soil carbon, aggregation and microbial community was examined after three growing seasons. The six cropping systems involved corn, sorghum, soybean, red clover, timothy and bare fallow, and differed in terms of tillage practice, type of crop (perennial versus annual) and type, amount and quality of carbon inputs to soil. Soil structure under sorghum and timothy differed from that of bare soil. Soil total carbon content exceeded that of fallow only under the two perennial cropping systems. Microbial community size and activity, arbuscular mycorrhizal fungi, bacteria and Gram(-) bacteria were positively correlated with total carbon, mean weight diameter (MWD) of aggregates and abundance of macroaggregates, and negatively correlated with abundance of microaggregates. Fungi were positively correlated with MWD of aggregates and negatively correlated with abundance of microaggregates. Root biomass and lignin content had the greatest effects on the size and proportion of microbial groups correlated with soil structure and carbon content. The relationships between microbial community structure and soil structure, and between microbial community structure and plant material chemical characteristics were further explored with an experiment where the dynamics of soil microbial community structure and changes in soil structure during the decomposition process of soybean, corn, timothy and red clover materials were monitored for 28 weeks. Up to 53% of the variation in microbial community composition could be explained by the quality of added materials and up to 24% of the variation in soil structure could be explained by the composition of the soil microbial community. The C/N, hemicellulose, soluble C and fiber content of plant materials had the greatest effect on the composition of the microbial community. High soluble C and hemicellulose content increased the proportion of Gram(-) bacteria and decreased the proportion of Gram(+) bacteria, respectively, while high C/N and fiber content both increased the fungi-to-bacteria ratio. The concentration of lignin affected the nutritional and physiological status of bacteria. Fungi and Gram(-) bacteria had the greatest effect on soil structure, primarily at the macroaggregation level. A relationship between Gram(-) bacteria and macroaggregate abundance was also observed for the first time. It appears

that Gram(-) and Gram(+) bacteria contribute differently to the process of aggregation, which is consistent with the preferential role of Gram(-) bacteria at decomposing fresh organic material. This research suggests that the composition of the microbial community is an important determinant of soil structural quality. It emphasizes the importance of the root system as a contributor to the soil carbon pool and as a driver of microbial community structure with ramifications into soil aggregation and soil carbon turnover.

Résumé

L'effet de six systèmes de culture sur le carbone, l'agrégation et la communauté microbienne du sol a été évalué après trois saisons de croissance. Les systèmes de culture comprenaient le maïs, le sorgho, le soya, le trèfle, la fléole des prés ainsi que des parcelles en jachère et étaient caractérisés par différents types de travail du sol et de plantes (pérennes versus annuelles) et par la nature, quantité et qualité des apports de carbone au sol. Comparativement à la jachère, la structure du sol a été affectée par les systèmes de culture impliquant le sorgho et la fléole des prés et le carbone total du sol n'a été augmenté que sous les plantes pérennes. La taille et l'activité de la communauté microbienne ainsi que les champignons arbusculaires mycorhiziens, les bactéries en général et les bactéries à Gram négatif ont été positivement corrélés au diamètre moyen des agrégats et à la proportion de macroagrégats du sol, mais négativement corrélés à la proportion de microagrégats. Les champignons étaient positivement corrélés au diamètre moyen des agrégats et négativement corrélés à la proportion de microagrégats. La biomasse racinaire et le contenu en lignine des racines ont eu le plus d'effet sur la taille et la proportion des groupes microbiens corrélés à la structure et à la teneur en carbone du sol. La relation entre la structure du sol et la composition de la communauté microbienne ainsi que le lien entre la qualité des matières végétales et la structure de la communauté microbienne ont été examinés davantage lors d'une expérience en laboratoire. Du sol et des tissues de maïs, de soya, de trèfle et de fléole des prés ont été incubés pendant 28 semaines au cours desquelles la variation dans la structure des communautés microbiennes et dans la structure du sol a été examinée. Jusqu'à 53% de la variation de la composition microbienne a pu être expliquée par la qualité de matière végétale ajoutée au sol et jusqu'à 24% de la variation de la structure du sol a pu être expliquée par la composition de la communauté microbienne. Le rapport C/N et les concentrations d'hémicellulose, de carbone soluble et de fibre de la matière végétale ajoutée ont eu un effet important sur la composition de la communauté microbienne; un contenu élevé en carbone soluble et hémicellulose augmentant l'abondance des bactéries à Gram négatif et diminuant celle des bactéries à Gram positif du sol, respectivement, tandis qu'un contenu en fibre et un rapport C/N élevés augmentaient le rapport champignons/bactéries. Le

contenu en lignine a affecté le statut nutritionnel et physiologique des bactéries du sol. Les champignons et les bactéries à Gram négatif ont eu l'effet le plus important sur la structure du sol, principalement au niveau des macroagrégats. Cette relation entre les bactéries à Gram négatif et les macroagrégats, rapportée pour la première fois, fut aussi observée dans l'essai en champs. Cette relation suggère une implication différente des bactéries à Gram négatif et des bactéries à Gram positif dans le processus d'agrégation du sol. Ceci est en accord avec l'utilisation préférentielle de matière organique fraîche comme source d'énergie par les bactéries à Gram négatif. Cette étude suggère que la composition de la communauté microbienne du sol pourrait jouer un rôle important dans la détermination de la qualité structurale du sol et souligne l'effet déterminant du système racinaire sur le niveau de carbone du sol, tant au niveau des flux de carbone que de son influence sur la communauté microbienne et l'agrégation du sol.

Table of content

List of Tables

List of Figures

List of Appendices

Acknowledgements

I would like to thank the following persons:

Dedication

This work is dedicated to my mother, Simone Caniquitte. Without her, I could not go through this journey. Thank you for teaching me autonomy and persistence, for the unconditional support and inspiration.

Contribution of Authors

The manuscripts included in this thesis as Chapters 3 to 5 will be submitted for publication in scientific journals; the candidate and his supervisors, Dr. James Fyles, Dr. Donald Smith and Dr. Chantal Hamel, will appear as co-authors on the three papers. The candidate was responsible of conducting the research, analyzing the data and preparing the manuscripts. Dr. James Fyles, Dr. Chantal Hamel, and Dr. Donald Smith provided general guidance and editorial revisions throughout the entire process.

Chapter 1

General introduction

1.1 PROBLEMATIC:

The concentration of greenhouse gases (GHG) in the atmosphere has increased substantially since the industrial revolution and concern about the enhanced greenhouse effect has prompted international action to reduce GHG emissions. Deforestation, conversion of forests, grasslands, and wetlands to cropland, and other agricultural activities have been responsible for a part of the total increase in the concentration of GHG through conversion of organic matter to CO_2. The agriculture sector contributes for about 50% and 70% of anthropogenic methane (CH_4) and nitrous oxide (N_2O) emissions, respectively and about 5% of anthropogenic carbon dioxide (CO_2) emissions (Cole, 1996; Mosier et al., 1998).

Conversion of native ecosystems to agriculture almost invariably results in a net loss of soil carbon. The rapid increase in the world's agricultural area over the past 300 years has been responsible for large CO_2 emissions. Because these agricultural soils are now C-depleted, they represent a potential CO_2 sink if part of the lost carbon can be regained. While there is strong evidence that agricultural soils can be a net sink for CO_2, the eventual size of that potential sink is uncertain. However, increasing the soil organic matter contents of Canadian agricultural soils by about 1% (from current average levels of 4 to 5%) would store in the order of 1 billion tonnes of carbon. Different crops and soil management practices associated with their cultivation may differ in their capacity to sequester large amounts of carbon. Soil organic matter decomposition and soil aggregation appear to be key points when it comes to storing carbon in soil. A good understanding of these processes and the factors by which they are influenced is important if we want to adopt management practices that will be effective in reducing or at least maintain the level of atmospheric greenhouse gases.

1.2 THE OBJECTIVES OF THE RESEARCH

The general objective of this research was to understand the mechanisms by which carbon sequestration into soil can be maximized and more specifically to investigate the poorly explored link between the microbial community composition and soil structure.

The specific objectives of the study were:

1) To assess the effect of cropping systems differing in crop species, carbon input to soil (quality and quantity) and soil management practices (surface tillage or no-tillage) on soil carbon.

2) To explore the relationships between soil structure, carbon content and microbial community composition, size and activity.

3) To identify the cropping system characteristics (soil management practices and crop characteristics) with the greatest influence on soil microbial community composition.

4) To study how changes in microbial community structure affect soil structure.

5) To determine how the initial chemical characteristics of decomposing plant material influence the composition of the soil microbial populations.

1.3 THE STRUCTURE OF THE THESIS

Chapter 2 presents a review of the main factors affecting soil organic carbon levels in soil. Chapter 3 presents a multi-cropping system field experiment designed to assess the effect of different crop species, carbon input (quality and quantity) and soil management practices (surface tillage or no-tillage) on soil carbon levels, soil structure and microbial community. The relationships existing between soil structure, carbon content and soil microbial community size, activity and composition are reported in Chapter 3. Chapter 4 presents an incubation experiment designed to explore the link between the microbial community and soil structure as influenced by plant material decomposition. In Chapter 5, the relationship between the chemical characteristics of plant material and the composition of the microbial community during the decomposition process is reported. Chapter 6 reviews the research objectives, the main findings of the research and provides a general conclusion.

REFERENCES

Cole, C.V. 1996. Agricultural options for mitigation of GHG emissions. In Climate Change 1995, Second Assessment Report. Cambridge University Press Publ. Chapter 23: 745–771.

Mosier, A.R., Duxbury, J.M., Freney, J.R., Heinemeyer, O. and Minami, K. 1998. Mitigating agricultural emissions of nitrous oxide. Climate Change 40: 7–39.

Chapter 2

Literature review

2.1 INTRODUCTION

The largest terrestrial pool of carbon is soil organic matter (SOM) (Carter, 1996). Recently, the search for ways to mitigate accumulation of greenhouse gases that leads to climate change has pointed out the possibility of increasing storage of carbon from atmospheric CO_2 in the soil, mainly by the use of soil management practices that increase the synthesis and/or retention of SOM (Carter, 1996).

One of the most important functions of SOM is to provide metabolic energy for the biological processes of the soil (Carter, 1996). During photosynthesis, CO_2 is taken from the atmosphere and converted into a wide variety of carbon compounds. The carbon of those compounds is then deposited on or in the soil where it is used as building material, released as metabolic products or respired as CO_2 by the organisms of the soil (Carter, 1996). Thus, SOM-carbon levels are determined by the balance of carbon inputs as plant residues and carbon losses through organic matter decomposition. The concentration of atmospheric CO_2 can be lowered by reducing SOM decomposition, thus reducing CO_2 emissions from soil respiration, or by enhancing CO_2 assimilation from the atmosphere via photosynthesis.

2.2 FACTORS INFLUENCING ORGANIC MATTER LEVELS IN SOIL

The SOM includes plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and the substances synthesized by the total population of soil organisms (Gregorich et al., 2001a). Soil organic matter levels depend on the balance between primary productivity and the rate of decomposition. Thus, SOM levels are influenced by the amount of above-ground residue returned to the soil, the root biomass and amount of root exudates produced by the plant, as well as the size of the microbial population supported by the plant.

2.2.1 ORGANIC MATTER PRODUCTION

2.2.1.1 Above-ground production

Soil organic matter content generally increases with increasing amounts of residues returned to the soil (Larson et al., 1972; Rasmussen et al., 1980; Pikul and Allmaras, 1986; Power et al., 1998; Duiker and Lal, 1999). Application of nutritive amendments, such as fertilizers, increases input of carbon to soil by increasing yields and, consequently, the amount of residues returned to the soil. Several studies examining the impact of fertilizer additions to soil have shown that fertilization enhances crop production and carbon inputs. Many experiments have shown a positive effect of N additions on the soil carbon balance through soil acidification reducing microbial activity (Thurston et al., 1976), depletion of soil water by larger plants (Andrén, 1987) and formation of recalcitrant humic substances in soil (Fog, 1988).

However, some studies have shown that increasing the amount of residues returned to the soil does not always result in increased organic matter and that factors other than the absolute amount of carbon input to soil may also influence SOM cycling. In a study by Buyanovsky and Wagner (1987), wheat contributed more carbon to SOM than corn although estimated annual carbon inputs were up to three times higher for corn than for wheat. Gregorich et al. (2001b) measured changes in soil carbon after 35 years of corn monoculture, legume-based rotation and continuous grass, and found that soil under monoculture corn and rotation had respectively 33 and 17% less carbon than the soil under continuous grass. Cumulative residue carbon and soil carbon were not correlated suggesting that qualitative differences in crop residues can have a greater effect on carbon storage than quantitative differences.

2.2.1.2 Root biomass and root exudates production

The root system affects SOM accumulation. Its biomass and exudates, which are continuously released in soil, are important sources of SOM. Plants commonly lose 10 to 30% of their photosynthates into the soil through passive and active root excretions, root cap cells sloughing off, and epidermal cell senescence (Bowen and Rovira, 1999). This

constitutes a considerable carbon input. A study conducted by Gale and Cambardella (2000) suggested that the carbon input from roots exceeds that from above-ground plant residues. Similar results were obtained by Balesdent and Balabane (1996) who reported that below-ground corn residue contributed 21 g carbon m^{-2} y^{-1} more to SOM than above-ground corn residues, although the above-ground biomass inputs were 193 g carbon m^{-2} y^{-1} higher than below-ground inputs. By compiling the results of several long-term studies, Bolinder et al. (1999) estimated that 12.2% of corn shoot residues are retained as soil organic matter compared to 21.1% for roots.

In a Chicot fine sandy loam, corn and switchgrass had similar total above-ground biomass carbon, but switchgrass had a higher root biomass and root/shoot ratio. Switchgrass contribution to soil carbon exceeded that of corn by 3 t/ha per year, even though all above-ground parts were removed from the field when switchgrass was harvested (Zan et al., 2001). Balesdent and Balabane (1996) noted that the differences between root and shoot contribution to soil organic matter could be explained by greater physical protection of root material by closer interaction with the soil matrix, and by the chemical composition of roots and root-derived products, which have higher lignin to nitrogen ratios than above-ground residues. Higher phenolic and lignaceous compounds in roots and relatively higher soluble carbon concentration in above-ground plant parts have also been proposed to explain the large contribution of roots to soil carbon (Bolinder et al., 1999).

2.2.2 ORGANIC MATTER DECOMPOSITION

Once deposited on or in the soil, plant residues (above and below-ground) and root exudates undergo decomposition. During the process of decomposition part of the carbon contained in this organic matter (OM) (residues and exudates) will be respired as CO_2, part of it will be assimilated by the microbial biomass and the remaining part will be humified, converted by the microbes and by condensation reactions among microbial products to more stable compounds. The decomposition process will determine how much carbon will be lost to the atmosphere and how much of it will remain in the soil.

2.2.2.1 FACTORS INFLUENCING OM DECOMPOSITION

Several factors affect OM decomposition. These include the degree of physical and chemical protection of the OM within aggregates and the chemical composition of the residues.

2.2.2.1.1 AGGREGATION

Much of the OM within aggregates is physically and/or chemically protected from decomposition (Carter, 1996). Chemical protection can be provided by the interaction of OM with soil mineral particles. Within aggregates, OM can be located in pores small enough to exclude certain microbes thus providing OM with physical protection. The small pores also reduce the diffusion of O_2, reducing microbial activity and, thus, decomposition.

In temperate-area soils, the process of aggregation is complex and involves several factors, including the action of plant roots, microbial products and processes mediated by arbuscular mycorrhizal fungi (AMF) (Tisdall and Oades, 1982; Miller and Jastrow, 1990; Tisdall, 1991). Agricultural practices such as tillage can have an important effect on the stability of soil aggregates.

a. Effect of plant roots on aggregation

Plant roots have direct and indirect effects on soil aggregation. According to a model proposed by Tisdall and Oades (1982), highly stable microaggregates are brought together and temporarily stabilized by the enmeshing effect of plant roots and fungal hyphae to form macroaggregates. Roots also exert an indirect effect on aggregate stability by stimulating the microbial biomass, which produces metabolic products acting as binding agents stabilizing aggregates (Tisdall and Oades, 1979; Jastrow et al., 1998; Gupta and Germida, 1988; Elliott and Coleman, 1988; Tisdall, 1991).

Differences in soil aggregate stability under different crops has been attributed to differences in the arbuscular mycorrhizal fungi (AMF) populations supported by these different crops (Tisdall and Oades, 1979) as well as qualitative differences in organic material released from living roots (Reid and Goss, 1981).

Shamoot et al. (1968) showed that the amount of organic carbon released by root systems is closely related to total root mass. Stone and Buttery (1989) have also found that the root dry weight of nine forage plants was positively correlated with soil organic carbon and wet aggregate stability. Higher inputs of organic carbon may support a larger microbial population producing exocellular polysaccharides, which results in greater aggregate stability.

Greater root mass and length and greater microbial biomass do not always result in greater aggregate stability. In a greenhouse experiment involving legume and non–legume crops, root mass and length, microbial biomass carbon, water-extractable carbohydrates and aggregate stability followed a similar trend when non-legume crops were considered (Haynes and Beare, 1997). But while Italian ryegrass had larger root mass and root length and was associated with larger soil microbial biomass carbon than white clover and lupin, aggregate stability was largest under lupin. The soil under ryegrass and white clover had a similar level of aggregate stability.

b. Effect of fungi and AMF on aggregation

It is now well established that AMF and fungi in general are involved in aggregate formation and stabilization. Fungal hyphae affect aggregation by physically holding together soil particles and by producing polysaccharides that act as binding agents between soil particles.

AMF were found to produce a glycoprotein, called glomalin, during the process of root colonization (Wright et al., 1996). Wright and Upadhyaya (1998) have shown that the concentration of easily extractable immunoreactive glomalin in a range of soils is related to aggregate stability. A strong positive linear correlation has also been found between total glomalin and aggregate stability (Wright et al., 1999; Wright and Anderson, 2000) and between total glomalin and soil carbon (Wright and Upadhyaya, 1996; Wright and Upadhyaya, 1998; Rillig et al., 2003). In contrast, a study conducted on undisturbed soils with high organic matter showed no particularly strong linkage between glomalin and aggregate stability (Franzluebbers et al., 2000).

Glomalin production can differ among plant species (Rillig et al., 2002). Glomalin has also been found to respond to land-use change and management practices. Rillig et al.

(2003) reported higher concentrations of glomalin under native forests as compared to agricultural and afforested soil. Wright et al. (1999) found that the glomalin content was higher 2-3 years after transition from conventional plough tillage to no-till corn; in addition, the amount of glomalin in a nearby soil under grass for 15 years was 45% higher than under a no-till annual cropping system. Similar results were obtained by Wright and Anderson (2000), where glomalin concentration was highest under perennial grass, lowest under spring cereal and intermediate under crop rotations.

c. Effect of microbial decomposition on aggregation

The processes of organic matter decomposition and aggregation are interrelated. Soil aggregates are formed around decomposing organic material through the binding effect of microbial polysaccharides excreted by microbial cells during the process of decomposition. The decomposing organic matter, thus becomes the center of a building aggregate. The biochemistry of a residue influences its decomposition rate and consequently the production of microbial products acting as binding agents for aggregate stabilization. In general, materials that are more easily decomposed will have a rapid but transient effect on aggregate stability while materials that decompose more slowly will have a long-term and more persistent effect (Martens, 2000). In a study conducted by Martens (2000), addition of seven different types of organic residues increased the mean weight diameter (MWD) of aggregates after 84 days of incubation when compared to the control. Residues higher in phenolic acid content (e.g., corn) were most effective at promoting MWD and also resulted in less loss of soil organic carbon, as compared to residues lower in phenolic acids (e.g., soybean and red clover). In addition, there were no differences in the formation of aggregates, between high and low C/N residues (Bossuyt et al., 2001).

d. Effect of tillage on aggregates

Soil aggregation, a reflection of SOM storage, is influenced by land use, including soil and crop management practices (Carter, 1996). Management practices such as tillage can have an important influence on SOM storage. Disruption of soil aggregates that

protect OM from decomposition, stimulation of microbial activity through enhanced aeration, and mixing of fresh residues into the soil where conditions for decomposition are more favourable than on the surface are some of the mechanisms by which tillage can promote soil carbon loss. Dick (1983) reported an increase from 12-25% in organic carbon under no-tillage, as compared to conventional tillage. Similar results were obtained by Blevins et al. (1977, 1983) and Dalal (1989). Arshad et al. (1990) found that the carbon and nitrogen contents of a no-till Donnelly silty loam soil were 26% higher than those of conventionally-tilled soil and that no-tilled systems could improve SOM resistence to degradation. Field studies have shown increases in macroaggregate stability with reduced tillage, especially with no-till (Kladivko et al., 1986; Beare et al., 1994). Greater aggregate stability was measured under minimum tillage and no-tillage as compared to conventional tillage and after two or more years of growing grain sorghum as compared to soybean (Bruce et al., 1990).

2.2.2.1.2 CHEMICAL COMPOSITION OF THE RESIDUE

Crop residue biochemistry has an effect on its decomposition and consequently on carbon persistence in soil. Different crop residues generally contain the same types of organic compounds, cellulose, hemicellulose, lignin, polyphenols, starches, lipids, proteins, but the proportion of each class is often different. Thus, the inherent decomposability of crop residues varies because of their physicochemical characteristics (Andrén and Paustian, 1987).

Many incubation experiments have been conducted in attempts to predict the rate of litter decomposition, and several predictive equations have been proposed using various ratios of carbon, nitrogen, lignin and polyphenols.

a. Effect of C/N ratio on residue decomposition

Carbon-to-nitrogen ratio was established, around the 1920s, as a general index for litter quality. Cumulative CO_2 evolution appears to be related to the C/N ratio of the residue only during a relatively short period following introduction of the residue into the soil. Bossuyt et al. (2001) found differences in cumulative respiration only during the first 10 days of incubation when comparing high (C/N = 20) and low quality residues (C/N = 108). Similar results were obtained by Bremer et al. (1991). The initial rate of CO_2

evolution was much faster when lentil green manure (C/N = 9) was incubated, as compared to lentil or wheat straw (C/N = 36 and 80 respectively). Initial decomposition rates have been associated with breakdown of the carbohydrate and amino-acid fractions of the residue (Corbeels et al., 2000; Martens, 2000).

b. Effect of lignin on residue decomposition

The most commonly used chemical measures of litter quality are lignin and certain polyphenolic substances (Paustian et al., 1997b). Depending on the residues, some factors can be better indicators of their quality than others. For instance, in residues containing less than 10-15% lignin, a C/N ratio of 50-100 is a reasonable predictor of decomposition rate. Over the same C/N range, some litters have higher lignin contents than others. Lignin is not only refractory to microbial enzymes but also protects cell wall polysaccharides from microbial attack. Thus, for residue with high lignin contents, the lignin/N ratio may be a better indicator of carbon availability to microorganisms than lignin content (Heal et al., 1997). Melillo et al. (1982) and Tian et al. (1992) have shown a correlation between lignin content and short-term decomposition rate of fresh residues.

In a study by Trinsoutrot et al. (2000), the carbon mineralization of 47 residues was monitored over 168 days in an attempt to establish relationships between the kinetics of carbon decomposition under nonlimiting nitrogen conditions and the biochemical characteristics of the residues. Residue decomposition was only slightly affected by their C/N ratio at the start of incubation. There was a strong positive relationship between the soluble fractions of the residues and carbon mineralization at the beginning of decomposition. Similar results were obtained by Cogle et al. (1989), Collins et al. (1990), Herman et al. (1977) and Reinertsen et al. (1984). In the later stages of decomposition, the carbon mineralized from the residues was positively correlated with polyphenols and negatively correlated with the lignin and hemicellulose contents of the residues.

c. Effect of component interactions on residue decomposition

Some studies have pointed out that there is an interaction among residue components that affects decomposition. Collins et al. (1990) found that CO_2 evolution from a mix of wheat residue components was 25% higher than the sum of the CO_2 evolved from

individual components. The stimulation of microbial activity and biomass by the higher quality components of the mix has been suggested as an explanation. Similar results were obtained by Quemada and Cabrera (1995) who studied the decomposition of leaves, stems and a mix of leaves and stems of oat, wheat and rye. Mixing leaves and stems increased the amount of CO_2 evolved by 5-8% as compared to the predicted values from isolated leaves or stems.

d. Effect of N addition on residue decomposition

Nitrogen is one of the most common factors limiting residue decomposition because of its strong influence on microbial biomass growth and turnover (Heal et al., 1997). Several studies have investigated the effect of added N on residue decomposition. Both positive and negative effects have been observed and the response appears to be dependent on residue quality. Bossuyt et al. (2001) found that respiration rates were slower for the first 10 days when wheat straw (C/N = 108) was incubated with added N as compared to incubation without N addition. Added N also reduced the amount of CO_2 evolved from soil incubated with lentil and wheat straw (Bremer et al. 1991). Contradictory results were obtained by Corbeels et al. (2000). In their incubation experiment, addition of N increased carbon mineralization from wheat straw (C/N = 67, soluble carbohydrates = 12.7%) during the first two weeks, whereas no effect on carbon mineralization was observed following addition of N to sunflower stalks (C/N = 36, soluble carbohydrates = 25.7%). Söderström et al. (1983) suggested that inhibition of microbial activity by decreased pH following addition of mineral N might be responsible for the negative response of carbon mineralization after N addition. Inhibition of lignolytic enzymes has also been suggested as another possible explanation (Keyser et al. 1978).

e. Effect of residue size and location on decomposition

The size of incubated residues may affect their decomposition rate. Different types of responses have been observed and they vary according to residue quality. For high quality residue (low C/N ratio), all types of responses have been reported. Bremer et al. (1991) and Bending and Turner (1999) found that decreasing the size of the high quality

residue does not seem to affect decomposition. Angers and Recous (1997) reported an increase in carbon mineralization from rye residue (C/N = 9) with a decrease in residue size only during the first two days of incubation. The decomposition was then slower for finer rye residue. Jensen (1994) also observed that decreasing the size of low C/N residues slowed decomposition during the first 30 days of incubation. The decreased decomposition rate of finer residue particles with low C/N ratio has been attributed to the more intimate contact of the residue with soil mineral particles and subsequent protection of the residue from microbial attack (Jensen, 1994; Breland, 1994). In the case of low quality residues, different types of responses have also been observed. Decreasing the size of residues with low C/N ratio has increased (Bremer et al., 1991; Tarafdar et al., 2001) as well as decreased carbon mineralization in the early stages (Bending and Turner, 1999) or later stages of decomposition (Angers and Recous, 1997).

Reduced rate of decomposition has been observed when residues are left on the soil surface rather than mixed into the soil where conditions are more favourable for decomposition and contact between soil and residues is enhanced (e.g. Coppens et al., 2006; Seneviratne et al., 1998; Ghidey and Alberts, 1993; Beare et al., 1993).

2.3 ANNUAL VS PERENNIAL CROP EFFECTS ON CARBON STORAGE

Using crops that maintain or improve soil organic matter and soil structure is necessary in order to maintain or increase the soil carbon stock. Perennial crops have proven useful in this regard. The generally greater capacity of perennial crops to increase soil carbon could be a result of extended periods of active growth and more allocation of their biomass to below-ground structures, as compared to annual crops (Paustian et al., 1997a). This results in greater aggregate stability (Reid and Goss, 1981), in particular for grass roots, and higher microbial biomass under perennial than under annual species (Chantigny et al., 1996; Drury et al., 1991). In addition, with perennial crops the soil is not disturbed by tillage.

Several studies have shown that perennial forages have a beneficial effect on soil aggregation. Forage grass species generally produce a high ratio of below- to above-ground biomass and have a high root mass and length density which has been associated

with greater increases in MWD of aggregates, as compared to annual row crops (e.g., Haynes and Francis, 1993).

Angers (1992) compared the MWD of water stable aggregates and the percentage of aggregate > 2 mm under corn, fallow and alfalfa. The results showed that, after 5 years, there was no change with time under corn and fallow but both parameters increased under alfalfa. Perfect et al. (1990b), and Haynes and Francis (1993) and Chantigny et al. (1997) also found highest MWD and improved aggregate stability under perennial as compared to annual cropping systems after three growing seasons.

Perfect et al. (1990a) studied the effect of alfalfa, bromegrass, red clover and corn on dispersible clay (DC), wet aggregate stability (WAS), soil moisture and microbial biomass. Over the third growing season, the corn treatments (conventional tillage and zero tillage) had higher DC, lower WAS and higher soil moisture than the perennial forage treatments. Root length and weight were different among the cropping systems (corn < legumes < grass) and were positively correlated with microbial biomass. Water content at time of sampling was found to be the first predictor of soil structural stability, followed by microbial biomass as a second predictor.

Similar results were found in a study including corn, soybean, alfalfa, red clover, reed canary grass and orchard grass (Drury et al., 1991). Aggregate stability was positively correlated with microbial biomass and was lower under corn and soybean than forages. Microbial biomass and aggregate stability were not different under corn or soybean.

2.4 SOIL MICROORGANISMS EFFECT ON SOIL CARBON LEVELS.

Soil microorganisms play a critical role in soil functioning through their implication in many soil processes including carbon cycling, nitrogen cycling, and the building of soil aggregates. They directly affect soil organic carbon levels through the crucial role they play in the processes of soil organic matter decomposition and humification, and indirectly through their effect on soil structure and fertility, with ramifications on plant biomass production.

Soil microbial biomass carbon (MB-C) represents only 1-4% of total soil organic carbon (Anderson and Domsch, 1989; Sparling, 1992). It has a rapid turnover time of 1-3

years and is therefore usually regarded as a reservoir of plant nutrients rather than a sink to store carbon in soil (Jenkinson and Ladd, 1981; Schnurer et al., 1985).

Microbial communities dominated by fungi are thought to favour soil carbon accumulation. Compared to bacteria, fungi incorporate more carbon in their biomass and release less CO_2 per unit of decomposed substrate (Holland and Coleman, 1987). Fungal products are more recalcitrant to decay (Martin and Haider, 1979) and appear to benefit from more protection by soil aggregates than bacterial products (Simpson et al., 2004).

The production of glomalin by AMF hyphae could also favour soil carbon accumulation. The harsh treatment needed to extract the protein from soil suggests it is a stable compound resisting decomposition and that it can be very persistent in soil when stabilized. Rillig et al. (2001) estimated glomalin to have a turnover of 6 to 42 years, much longer than the turnover of AMF hyphae, which is assumed to be in the order of days to weeks. Incubation experiments have shown that glomalin decomposes more slowly than hyphae of mycorrhizal fungi. After 150 days of incubation, total glomalin declined by only 25% as compared to 60% for arbuscular mycorrhizae hyphal length (Steinberg and Rillig, 2003). Similar results were obtained by Rillig et al. (2003) when they incubated the A horizon of forest and agricultural soils. After 400 days, about 50% of the total glomalin remained. This suggests that contributions of mycorrhizae to soil carbon storage based on hyphal biomass in soil and roots may have been underestimated.

Soil microorganisms were already identified as one of the factors contributing to the building and stabilization of soil structure in the early 1900s. The physical enmeshment of soil particles by fungal hyphae and the binding properties of microbial polysaccharides are the main mechanisms by which soil microorganisms affect soil aggregation.

Organic matter decomposition is essential to the processes of soil aggregation and stabilization of soil carbon. During decomposition, soil microbes produce polysaccharides that bind soil particles, which forms aggregates around decomposing organic matter. At the center of the aggregate, the decomposing organic material and its associated microbes are physically protected from decomposition by other soil organisms and soil mineral-organic matter-microorganisms associations are formed. When microorganisms die, soil mineral-organic matter complexes remain and protect the organic matter against further decomposition (Kandeler et al., 2005). During the process

of decomposition, organic materials are transformed toward increased complexity and stability. Microbial decomposition leads to the process of humification and the production of humic substances with high stability and slow turnover rate.

Although the link between soil microorganisms and soil aggregation is not questionable, the relationship between soil structure and microbial community appears to be complex and is still not fully understood. Microbial biomass and polysaccharides have both been reported to show either strong, weak or no correlations with soil aggregates. (e.g., Perfect et al., 1990a; Drury et al., 1991; Angers et al., 1992; Carter et al., 1994; Degens et al., 1994; Chantigny et al., 1997).. A good understanding of the factors affecting the soil microbial community and better understanding of the relationships between microbial community and soil structure would help design to management strategies to improve carbon sequestration and storage into soil.

REFERENCES

Anderson, T.H. and Domsch, K.H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biology and Biochemistry 21:471-479.

Andrén, O. 1987. Decomposition in the field of shoots and roots of barley, lucerne and meadow fescue. Swedish Journal of Agricultural Research 17:113-122.

Andrén, O. and Paustian, K. 1987. Barley straw decomposition in the field – a comparison of models. Ecology 68:1190-1200

Angers, D.A. 1992. Changes in soil aggregation and organic carbon under corn and alfalfa. Soil Science Society of America Journal 56: 1244-1249

Angers, D.A., Pesant, A. and Vigneux, J. 1992. Early cropping-induced changes in soil aggregation, organic matter and microbial biomass. Soil Science Society of America Journal 56:115-119.

Angers, D.A., Recous, S. 1997. Decomposition of wheat straw and rye residues as affected by particle size. Plant and Soil 189:197-203

Arshad, M.A., Schnitzer, M., Angers, D.A. and Ripmeester, J.A. 1990. Effects of till vs. no-till on the quality of soil organic matter. Soil Biology and Biochemistry 22: 595-599.

Balesdent, J. and Balabane, M. 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil Biology and Biochemistry 28:1261-1263.

Beare, M.H., Hendrix, P.F. and Coleman, D.C. 1994. Water-stable aggregates and organic matter fractions in conventional and no-tillage soils. Soil Science Society of America Journal 58:777-786.

Beare, M.H., Pohlad, B.R., Wright, D.H. and Coleman, D.C. 1993. Residue placement and fungicide effects on fungal communities in conventional and no-tillage soils. Soil Science Society of America Journal 57: 392-399.

Bending, G.D. and Turner, M.K. 1999. Interaction of biochemical quality and particle size of crop residues and its effect on the microbial biomass and nitrogen dynamics following incorporation into soil. Biology and Fertility of Soils 29:319-327.

Blevins, R.L., Thomas, G.W. and Cornelius, P.L. 1977. Influence of no-tillage and fertilization on certain soil properties after five years of continuous corn. Agronomy Journal 68:383-386.

Blevins, R.L., Thomas, G.W., Smith, S.M., Frye, W.W. and Cornelius, P.L. 1983. Changes in soil properties after 10 years continuous non-tilled and conventionally tilled corn. Soil Tillage Research 3:135-145.

Bolinder, M.A., Angers, D.A., Giroux, M. and Laverdière, M.R. 1999. Estimating C inputs retained as soil organic matter from corn (*Zea Mays L.*). Plant and Soil 215:85-91

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R. and Paustian, K. 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16: 195-208.

Bowen, G.D. and Rovira, A.D. 1999. The rhizosphere and its management to improve plant growth. Advances in Agronomy 66: 1-249.

Breland, T.A. 1994. Enhanced mineralization and denitrification as a result of heterogenous distribution of clover residues in soil. Plant and Soil 166:1-12.

Bremer, E., Van Houtum, W. and, Van Kessel, C. 1991. Carbon dioxide evolution from wheat and lentil residues as affected by grinding, added nitrogen and the absence of soil. Biology and Fertility of Soils 11: 221-227.

Bruce, R.R., Langdale, G.W. and Dillard, A.L. 1990. Tillage and crop rotation effect on characteristics of a sandy surface soil. Soil Science Society of America Journal 54: 1744-1747.

Buyanovsky, G.A. and Wagner, G.H. 1987. Carbon transfer in a winter wheat (*Triticum aestivum*) ecosystem. Biology and Fertility of Soils 5:76-82.

Carter, M.R. 1996. Analysis of soil organic matter storage in agroecosystems. In: Structure and organic matter storage in agricultural soils. Edited by M.R. Carter and B.A. Stewart. Advances in Soil Science. CRC Press Inc.

Carter, M.R., Angers, D.A. and Kunelius, H.T. 1994. Soil structural form and stability and organic matter under cool season perennial grasses. Soil Science Society of America Journal 58:1194-1199.

Cogle, A.L., Saffigna, P.G., and Strong, W.M. 1989. Carbon transformation during wheat straw decomposition. Soil Biology and Biochemistry 21:367-372.

Collins, H.P., Elliot, L.F., Rickman, R.W., Bezdicek, D.F., and Papendick, R.I. 1990. Decomposition and interaction among wheat residue components. Soil Science Society of America Journal 54:780-785.

Coppens, F., Garnier, P., Merckx, R. and Recous, S. 2006. Soil moisture carbon and nitrogen dynamics following incorporation versus surface application of labelled residues in soil columns. European Journal of Soil Science 57: 894–905.

Corbeels, M., Hofman, G. and Van Cleemput, O. 2000. Nitrogen cycling associated with the decomposition of sunflower stalks and wheat straw in a Vertisol. Plant and Soil 218: 71-82.

Dalal, R.C. 1989. Long-term effects of no-tillage, crop residue, and nitrogen application on properties of a Vertisol. Soil Science Society of America Journal 53:1511-1515

Degens, B.P., Sparling, G.P. and Abbott, L.K. 1994. The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long term clover-based and grass pastures. European Journal of Soil Science 45: 459-468.

Dick, W.A. 1983. Organic carbon, nitrogen and phosphorus concentrations and pH in soil profiles as affected by tillage intensity. Soil Science Society America Journal 47:102-107.

Duiker, S.W., and Lal, R. 1999. Crop residue and tillage effects on carbon sequestration in a Luvisol in central Ohio. Soil and Tillage Research 52: 73-81

Elliott, E.T. and Coleman, D.C. 1988. Let the soil work for us. Ecological Bulletins 39:23-32

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews of the Cambridge Physolophical Society 63:433-462

Franzluebbers, A.J., Wright, S.F. and Stuedemann, J.A. 2000. Soil aggregation and glomalin under pastures in the southern Piedmont, USA. Soil Science Society of America Journal 64:1018-1026

Gale, W.J. and Cambardella, C.A. 2000. Carbon dynamics of surface residue– and root-derived organic matter under simulated no-till. Soil Science Society of America Journal 64:190-195

Ghidey, F. and Alberts, E.E. 1993. Residue type and placement effects on decomposition: field study and model evaluation. Transactions of the ASAE 36: 1611-1617.7.

Gregorich, E.G., Turchenek, L.W., Carter, M.R. and Angers, D.A. (Eds) 2001a. Soil and Environmental Dictionary. CRC Press LLC

Gregorich, E.G., Drury, C.F. and Baldock, J.A. 2001b. Changes in soil carbon under long-term maize in monoculture and legume-based rotation. Canadian Journal of Soil Science 81: 21-31.

Gupta, V.V.S.R. and Germida, J.J. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Bioliogy and Biochemistry 20:777-786.

Haynes, R.J. and Beare, M.H. 1997. Influence of six crop species on aggregate stability and some labile organic matter fractions. Soil Biology and Biochemistry 29: 1647-1653.

Haynes, R.J. and Francis, G.S. 1993. Changes in microbial biomass, soil carbohydrates and aggregate stability induced by growth of selected crop and forage species under field conditions. Journal of Soil Science 44:665-675.

Heal, O.W., Anderson, J.M. and Swift, M.J. 1997. Plant litter quality and decomposition: An historical overview. In: Driven by nature: plant litter quality and decomposition. Edited by G. Cadisch and K.E. Giller. CAB International.

Herman, W.A., McGill, W.B., and Dormaar, J.R. 1977. Effect of initial chemical composition on decomposition of roots of three grass species. Canadian Journal of Soil Science 57:205-215.

Holland, E.A. and Coleman, D.C. 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology 68: 425-433.

Jastrow, J.D., Miller, R.M. and Lussenhop, J. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology and Biochemistry 30:905–916.

Jenkinson, D.S. and Ladd, J.N. 1981. Microbial biomass in soil: Measurement and turnover. In: Paul EA, Ladd JN (eds) Soil Biochemistry, Vol 5. Marcel Dekker, New York, pp 415-471.

Jensen, E.S. 1994. Mineralization-immobilization of nitrogen in soil amended with low C/N ratio plant residues with different particle sizes. Soil Biology and Biochemistry 26: 519-521.

Kandeler, E., Stemmer, M., Gerzabek, M.H. 2005. Role of microorganisms in carbon cycling in soils. *In*: Soil Biology, Volume 3, Microorganisms in soils: Role in genesis and functions. F. Buscot and A. Varma (Eds.), Springer-Verlag Berlin Heidelberg, Germany.

Keyser, P., Kirk, T.K., Zeikus, J.G. 1978. Lignolytic enzyme system of *Phanerichaete chrysosporium*: Synthesis in absence of lignin in response to nitrogen starvation. Journal of Bacteriology 135:790-797.

Kladivko, E.J., Griffith, D.R. and Mannering, J.V. 1986. Conservation tillage effects on soil properties and yield of corn and soya beans in Indiana. Soil and Tillage Research 8: 277-287.

Larson, W.E., Clapp, C.E., Pierre, W.H. and Morachan, Y.B. 1972. Effects of increasing amounts of organic residues on continuous corn. II. Organic C, N, P and S. Agronomy Journal 64: 204-208.

Martens, D.A. 2000. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. Soil Biology and Biochemistry 32: 361-369.

Martin, J.P. and Haider, K. 1979. Biodegradation of ^{14}C-labeled model and cornstalk lignins, phenols, model phenolase humic polymers, and fungal melanins as influenced by a readily available carbon source and soil. Applied and Environmental Microbiology 38: 283-289.

Melillo, J.M., Aber, D.J. and Muratore, J.F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621-626.

Miller, R.M. and Jastrow, J.D. 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biology and Biochemistry 22:579-584.

Paustian, K., Collins, H. P. and Paul, E.A. 1997a. Management controls on soil carbon. In: Soil organic matter in temperate agroecosystems: long-term experiments in North America. Edited by Paul E.A. et al. Boca Raton: CRC Press pp15-49.

Paustian, K., Agren, G. and Bosatta, E. 1997b. Modeling litter quality effects on decomposition and soil organic matter dynamics. In: Driven by Nature: plant litter quality and decomposition (Eds Cadish & K.E. Giller), CAB International, Wallingford, UK, pp 313-336.

Perfect, E., Kay, B.D., Van Loon, W.K.P., Sheard, R.W. and Pojasok, T. 1990a. Factors influencing soil structure stability within a growing season. Soil Science Society of America Journal 54: 173-179.

Perfect, E., Kay, B.D., Van Loon, W.K.P., Sheard, R.W. and Pojasok, T. 1990b. Rate of change in soil structural stability under forages and corn. Soil Science Society of America Journal 54:179-186.

Pikul, J.L., and Allmaras, R.R. 1986. Physical and chemical properties of a Haploxerol after fifty years of residue management. Soil Science Society of America Journal 50: 214-219.

Power, J.F., Koerner, P.T., Doran, J.W., and Wilhelm, W.W. 1998. Residual effects of crop residues on grain production and selected soil properties. Soil Science Society of America Journal 62: 1393-1397.

Quemada, M., and Cabrera, M.L. 1995. Carbon and nitrogen mineralized from leaves and stems of four cover crops. Soil Science Society of America Journal 59: 471-477.

Rasmussen, P.E., Allmaras, R.R., Rohde, C.R, and Roager, N.C., Jr. 1980. Crop residue influences on soil carbon and nitrogen in a wheat-fallow system. Soil Science Society of America Journal 44: 596-600.

Reid, J.B. and Goss, M.J. 1981. Effect of living roots of different plant species on the aggregate stability of two arable soils. Journal of Soil Science 32: 521-541.

Reinertsen, S.A., Elliot, L.F., Cochran, V.L. and Campbell, G.S. 1984. Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. Soil Biology and Biochemistry 16: 459-464.

Rillig, M.C., Ramsey, P.W., Morris, S. and Paul, E.A. 2003. Glomalin, an arbuscular-mycorrizal fungal soil protein, responds to land-use change. Plant and Soil 253: 293-299.

Rillig, M.C, Wright, S.F. and Eviner, V.T. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effect of five plant species. Plant and Soil 238:325-333.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F. and Torn, M.S. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant and Soil 233: 167-177.

Schnurer, J., Clarholm, M., Rosswall, T. 1985. Microbial biomass and activity in agricultural soil with different organic matter contents. Soil Biology and Biochemistry 17: 611-618.

Seneviratne, G., Van Holm, L.H.J., Kulasooriya, S.A. 1998. Quality of different mulch materials and their decomposition and N release under low moisture regimes. Biology and Fertility of Soils 26:136–140

Shamoot, S., McDonald, L., and Bartholomew, W.V. 1968. Rhizodeposition of organic debris in soil. Soil Science Society of America Proceedings 32:817-820.

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

Söderström, B., Baath, E., Lundgren, B. 1983. Decrease in soil microbial activity and biomass owing to nitrogen amendments. Canadian Journal of Microbiology 29: 1500-1506.

Sparling, G.P. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Australian Journal of Soil Research 30:195-207.

Steinberg, P.D. and Rillig, M.C. 2003. Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin. Soil Biology and Biochemistry 35:191-194.

Stone, J.A. and Buttery, B.R. 1989. Nine forages and the aggregation of a clay loam soil. Canadian Journal of Soil Science 69:165-169.

Tarafdar, J.C., Meena, S.C., Kathju, S. 2001. Influence of straw size on activity and biomass of soil microorganisms during decomposition. European Journal of Soil Biology 37: 157-160.

Thurston, J.M, Williams, E.D. and Johnston, A.E. 1976. Modern developments in an experiment on permanent grassland started in 1856: effects of fertilizers and lime on botanical composition and crop and soil analyses. Annales Agronomiques 27:1043-1082.

Tian, G., Kang, B.T. and Bussaard, L. 1992. Biological effect of plant residues with contrasting chemical compositions under humid tropical conditions. Decomposition and nutrient release. Soil Biology and Biochemistry 24:1051-1060.

Tisdall, J.M. 1991. Fungal hyphae and structural stability of soil. Australian Journal of Soil Research 29: 729-743.

Tisdall, J.M. and J.M. Oades. 1979. Stabilization of soil aggregates by the root systems of ryegrass. Australian Journal of Soil Research 29:729–743.

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

Trinsoutrot, I., Recous, S., Bentz, B., Linères, M., Chèneby, D. and Nicolardot, B. 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. Soil Science Society of America Journal 64: 918-926.

Wright, S.F., Franke-Snyder, M., Morton, J.B. and Upadhyaya, A. 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant and Soil 181:193-203.

Wright, S.F. and Upadhyaya, A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 1611: 575-586.

Wright, S.F. and Upadhyaya, A. 1998. A survey of soil for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil 198: 97-107.

Wright, S.F., Starr, J.L. and Paltineanu, I.C.1999. Changes in aggregate stability and concentration of glomalin during tillage management transition. Soil Science Society of America Journal 63:1825-1829.

Wright, S.F. and Anderson, R.L. 2000. Aggregate stability and glomalin in alternative crop rotations for the central great plains. Biology and Fertility of Soils 31:249-253.

Zan, C.S., Fyles, J.W., Girouard, P. and Samson, R. 2001. Carbon sequestration in perennial bioenergy, annual corn and uncultivated systems in southern Quebec. Agriculture Ecosystems and Environment 86: 135-144.

Chapter 3

Soil structure, carbon content and microbial community FAME: effect of different cropping systems and assessment of their relationships

3.1 INTRODUCTION

The search for ways to mitigate accumulation of the greenhouse gases causing climate change has led to consideration of increased carbon storage in soil through soil management practices that favour the maintenance or increase of soil organic matter (SOM) (Carter, 1996). SOM content generally increases with increasing amounts of residue returned to the soil (Larson et al., 1972; Rasmussen et al., 1980; Pikul and Allmaras, 1986; Power et al., 1998; Angers et al., 1999; Bissonnette et al., 2001; Duiker and Lal, 1999). However, some studies have shown that this is not always the case suggesting that factors other than the absolute amount of residue input may also influence the SOM cycling (Buyanovsky and Wagner, 1987; Huggins and Fuchs, 1997; Huggins et al., 1998; Reicosky et al., 2002). Qualitative differences in crop residues have been suggested to have a greater effect on carbon storage than quantitative differences (Gregorich et al., 2001). Soil management practices can also affect carbon storage. Adoption of conservation tillage practices, no tillage in particular, has been shown to promote soil carbon accumulation as compared to conventional tillage. West and Post (2002) used the results of 67 long-term experiments and estimated that conversion from conventional to no tillage can sequester an average of 57 $\pm$14 g C m^{-2} yr^{-1}. Tillage can induce a decrease in soil carbon by affecting SOM decomposition through several mechanisms including increased soil aeration, mixing of crop residue into the soil profile and disruption of soil aggregates, thus exposing previously protected organic matter to decomposition (Oades, 1984; Elliott, 1986; Beare et al., 1994).

Increasing the amount of organic matter entering the soil can be achieved quite easily through proper crop selection and management. However, once deposited on or in the soil, organic matter undergoes decomposition. Organic matter (OM) decomposition is

largely carried out by soil microorganisms and is affected mainly by residue size and quality as well as soil temperature, moisture, texture and structure. The processes of OM decomposition and soil aggregation dynamics are interrelated because OM is basic to the process of aggregation while OM within aggregates or micropores may be protected against degradation (Carter, 1996). During the early stage of decomposition, soil microorganisms process the easily available carbon and produce extracellular polysaccharides acting as cementing agents and required for the soil particles to combine into aggregates (Oades, 1984; Cheshire, 1979). Inside the newly formed aggregate, the OM is further decomposed and humified by its associated microbes and both the OM and associated microorganisms are physically protected from biodegradation by other soil organisms. Understanding the factors controlling organic matter decomposition and storage in soil is necessary to elaborate strategies that prevent further increase and possibly even reduce atmospheric CO_2 levels.

The role of plant roots and fungal hyphae on soil aggregation was suggested more than two decades ago (Tisdall and Oades, 1982) and has been demonstrated by many studies (e.g., Chantigny et al., 1997; Bossuyt et al., 2001). Arbuscular mycorrhizal fungi (AMF), and fungi in general, have a recognized effect on soil aggregation, particularly at the macroaggregate level. Soil physical entanglement by fungal hyphae (Tisdall and Oades 1982) and fungal polysaccharides (Puget et al., 1999) acting as binding agents plays a major role in aggregation. The contribution of fungi to soil structure has been suggested by many studies. As examples, in a study comparing the effect of annual and perennial cropping systems on aggregation, Chantigny et al. (1997) found strong correlation between mean weight diameter (MWD) of aggregates and fungal glucosamine after three growing seasons. Beare et al. (1997) found a 40% decrease in macroaggregates > 2 mm when no-till soils were treated with fungicide. Although the specific role of this protein is still a subject of debate (Feeney et al., 2004), AMF were found to produce a glycoprotein (glomalin) strongly correlated with aggregate stability (e.g., Wright et al., 1999; Nichols and Wright, 2005). Understanding the role of microorganisms in the formation of soil aggregates, and thus in the accumulation of carbon in the soil, is crucial.

Crop type and soil management have a large influence on soil microbial community structure and activity. Above and below-ground crop residues and root exudates provide soil microorganisms with metabolic energy and the quality and quantity of this OM influence the size, composition and activity of the microbial community. Tillage practices also influence the microbial community. Compared to no-till management, conventional tillage practices stimulate microbial activity through enhanced aeration and mixing of fresh residues into the soil where conditions for decomposition are more favourable than on the surface. No-till management may favour fungal biomass development, as compared to conventional tillage, where soil disturbance can have a detrimental effect on fungal hyphae (e.g., Wardle, 1995; Frey et al., 1999).

Among the methods available to study soil microorganisms, fatty acid methyl ester (FAME) analysis has been used to detect microbial community changes in response to management practices (Klug and Tiedje, 1993; Pankhurst, 1997), tillage (Drijber et al., 2000; Calderon et al., 2001), plant species (Ibekwe and Kennedy, 1999) and nitrogen fertilization (Bardgett et al., 1999). Fatty acid analysis provides insight into the microbial community composition since there is a correspondence between microbial species groups and the dominant fatty acids in their cell membranes. Polyunsaturated and normal saturated fatty acids longer than 20 carbons are associated with microeukaryotes; terminally-branched saturated fatty acids (iso and anteiso) are associated with Gram(+) bacteria while Gram(-) bacteria are associated with monounsaturated straight-chain fatty acids (White et al., 1996; Kunitsky et al., 2006). The FAME profile can be used to fingerprint the entire microbial community. Some specific FAMEs were shown to respond to environmental conditions such as carbon and O_2 limitations, high temperature and acidic conditions (Knivett and Cullen 1965; Allison et al., 2005) as well as substrate availability (Thomas and Batt, 1969; Bossio and Scow, 1998; Zelles et al., 1992).

This experiment included six cropping systems differing in terms of tillage practice, type of crop (perennial versus annual) and type, amount and quality of carbon inputs into the soil. We studied, after three growing seasons, the effect of the different cropping systems on soil structure, carbon content and microbial community size, activity and structure in order to (i) identify the relationships between soil structure, carbon content

28

and microbial community properties using FAME analysis, and (ii) determine which cropping system characteristics had the greatest influence on FAME profile and particularly FAME profiles having positive effects on soil aggregation and carbon content.

3.2 MATERIALS AND METHODS

3.2.1 Site description and management

This experiment was conducted in 2001-2003, at the Emile A. Lods Research Centre of the Macdonald Campus of McGill University in Ste-Anne-de-Bellevue, Quebec. The top 20 cm of the soil used was a Chicot fine sandy loam with 92.7% of sand, 2.1 of silt, and 5.2 % of clay. The site had been planted to soybean in 1998-1999, and was under fallow in 2000, with tillage used during the summer for weed control.

We compared six cropping systems, each system being characterized by their type of crop, tillage system and carbon input. The cropping systems involved three annual crops: corn (C), sorghum (Sg) and soybean (S), two perennial crops red clover (RC) and timothy (T), and bare fallow (BF). Some characteristics of the cropping systems are presented in Table 3.1. Corn (Pioneer 3921) was planted in rows spaced 75 cm apart. Each corn plot consisted of eight rows, 4 m long. Sorghum (sudangrass hybrid), red clover (Khun), timothy (Champ) and soybean (OAC Bayfield) were planted in rows spaced 20 cm apart. Each plot consisted of seven 4-m rows. The experiment was organized following a randomized complete block design with four blocks, and one replicate per treatment per block (n=4, Figure 3.1).

All crops were grown for three years on the same plots. The two perennial crops were not tilled during the three years of the experiment. The bare fallow plots and the plots with annual crops were tilled to prepare a seedbed and in the fall to 20 cm using a tractor (Kubota model BX1800) equipped with a B40C roto-tiller. Residues of corn, soybean and sorghum were returned to the soil. No above-ground biomass was returned to the soil after cutting red clover and timothy. Fertilizer was applied to sorghum and corn respectively as follows: ammonium nitrate was banded at seeding to provide 50 kg N ha^{-1} and 60 kg N ha^{-1} and an additional 100 kg N ha^{-1} and 120 kg N ha^{-1} was top dressed at the 6-leaf stage. For timothy, ammonium nitrate was banded at seeding to provide 60 kg N

ha^{-1} and the same rate of fertilizer was top-dressed the following seasons in late May. Soybean and red clover did not receive nitrogen fertilization. All plots received the recommended rates of phosphorus and potassium for this soil.

The first year, Basagan Forte was applied to soybean, post-emergence. The herbicides 75 DF Accent plus Agral and Embutox were used for corn and red clover, respectively. Weed control was by hand for sorghum and timothy. For the rest of the study, weed control was carried out by hand for all the crops. All crops were harvested by hand. After harvesting in the fall, above-ground crop residues (corn, sorghum and soybean) were chopped and incorporated into the soil by plowing to 20 cm. Dates of all field operations during this field study are shown in Table 3.2.

3.2.2 Soil sampling

Soil sampling was performed on the dates listed in Table 3.2. Twelve cores (2 cm diameter, 15 cm deep) were taken at random in each individual plot and combined to make one composite sample per plot. The samples were placed in a cooler containing ice from the site to the laboratory where they were homogenized and sub-sampled to be either air-dried (light fraction and total carbon determination), frozen (microbial biomass carbon and fatty acids extraction), or stored at 4°C for a maximum of five days (basal respiration determination).

3.2.3 Crop yield

The number of corn plants per plot was determined by counting the number of plant per row in two random rows in each plot. The corn cobs were collected, counted and fresh weight of stalks and cobs was recorded. The stalks were chopped with an Ohio sample chopper. A sub-sample was collected and weighed before drying for stover yield determination. The remaining chopped stalks were returned to the field. The cobs were removed from their husks, weighed and dried. Once dried, the cobs were shelled and reweighed for grain yield determination. Total yield was determined by adding grain and stover yields.

The number of soybean plants per plot was determined by counting the number of plants per row in two random rows in each plot. A sub-sample of 10 random plants per plot was collected for dry matter and yield determination. The beans of the remaining plants were harvested using a Wintersteiger Nursery Master Elite plot combine. The residues were returned to the field and the beans weighed for yield determination. The sub-samples of 10 plants per plot were dried and weighed. The beans were removed from the pods and their dry weight was recorded for grain yield determination. Total yield was determined by adding bean and stem yields.

The number of sorghum plants per plot was determined by counting the number of plants per row in two random rows in each plot. The stalks were chopped with an Ohio sample chopper and the fresh weight of stalks was recorded. A sub-sample was collected and weighed before drying for stover yield determination. The remaining chopped stalks were returned to the field.

Timothy and red clover were cut at 15 cm using a Forage plot harvester (Swift Machine & Welding Ltd.). When more than one cutting was done during the season, yields from each cutting were added to give total seasonal yield. Timothy and red clover above-ground biomass were not returned to the field after cutting, thus carbon inputs to soil in RC and T were essentially from roots.

All samples (cobs, stalks, beans, stems) were dried in a forced-draft oven at 70°C for a minimum of 48 hours or until constant weight before weighting.

3.2.4 Root biomass estimation

Root estimates were calculated for each year from 2001 to 2003 using the above-ground and/or grain biomass and the sum of all years was used as the estimated root biomass for three years.

The root biomass for corn, sorghum and soybean was estimated with the equation:

$$\text{Root biomass} = k \text{ (shoot + grain biomass)} \qquad [1]$$

where k = root/shoot ratio at maturity (Wilts et al., 2004). A root/shoot k value of 0.6, 0.21 and 0.23 was used for sorghum (Eneji et al., 2005; Kurdali, 2004), corn and soybean (Buyanovsky and Wagner, 1997), respectively.

The root biomass for timothy was estimated with the equation:

Root Weight Ratio (RWR) = root biomass/(root biomass + shoot biomass)   [2]

using a RWR of 0.32 (Bélanger et al., 2002; Brégard et al., 2001).

The root biomass for red clover was estimated with the equation:

Root/shoot ratio = root biomass/ shoot biomass   [3]

using a shoot/root ratio of 1.73 (Campbell et al., 1998).

3.2.5 Residue quality

Above-ground residue quality was determined on materials collected for biomass determination. Root samples were taken at harvest, before the incorporation of residue into the soil. Root sampling was done by digging with a shovel (20 cm diameter, 30 cm deep) around two random plant shoots in each plot. Collected samples were kept in the fridge for a maximum of 7 days before being washed free of soil with water. Roots and above-ground residues were dried in a forced-draft oven at 70°C for a minimum of 48 hours or until constant weight and ground to pass a 1-mm sieve. Total carbon and nitrogen were determined with an elemental Carlo Erba analyzer NCS 2500 (Thermo Quest Italia S.P.A., Rodano, Italy). Neutral and acid detergent fiber (NDF and ADF, respectively) were determined using the filter-bag technique and the ANKOM[200] fiber analyzer. Fiber content of plant materials was defined as being the neutral detergent fiber portion. Lignin content (acid detergent lignin) was determined using the beakers method as recommended by ANKOM Technology (ANKOM Technology, Fairport, New York, USA). The percentage of hemicellulose was determined as being the difference between % NDF and % ADF. Percentages of fiber, lignin and hemicellulose were expressed on a dry-mater basis.

3.2.6 Total carbon and light fraction carbon

Total carbon and light fraction carbon were determined on soil samples collected pre-harvest on September 18, 2003. The soil was air dried and crushed. Total carbon was determined with the elemental Carlo Erba analyzer NCS 2500 (Thermo Quest Italia S.P.A., Rodano, Italy). The light fraction was extracted from soil with a sodium iodide solution with a density of 1.7 g cm^{-3} as described by Gregorich and Ellert (1993). The isolated fraction was oven dried at 60°C, crushed and the carbon content was determined

using the elemental Carlo Erba analyzer NCS 2500 (Thermo quest Italia S.P.A., Rodano, Italy).

3.2.7 Aggregate-size distribution

Samples were taken pre-harvest in 2003 at four random locations in each plot. The top 10 cm of soil was collected with a shovel and handled as little as possible to minimize aggregate breakage. The soil samples were air dried for at least 7 days or until constant weight. Approximately 40 grams of soil were placed over the top of a nest of sieves (20.5 cm diameter) with openings of 4, 2, 1, 0.5 and 0.25 mm with a collecting pan at the bottom and a cover on top. The nest of sieves was placed on a Soil Test Engineering Test Equipment shaker, model CL-305 A, and the soil was dry sieved by shaking for 2 minutes at 120 oscillations per minute. The weight of the fraction remaining on each sieve was recorded and the results were expressed as grams of soil retained on the sieve per 100 g of total soil placed on the 4 mm sieve (% of total soil) on an air dry basis. The dry aggregate Mean Weight Diameter (MWD) was calculated as described by Kemper and Rosenau (1986). Macroaggregates were defined as the sum of aggregates greater than 0.25 mm in size and microaggregates were defined as the sum of aggregates smaller than or equal to 0.25 mm in size.

3.2.8 Microbial biomass carbon

The soil microbial biomass carbon (MBC) content was determined in 2003 at all sampling dates. After collection in the field, soil samples were frozen and thawed just prior to extraction using the chloroform fumigation-extraction method (Voroney et al., 1993) and a soil to extractant ratio of 1:4. Soil samples were analysed in duplicate. The organic carbon of the extracts was measured using the total organic carbon analyser TOC-V CSN and ASI-V (Shimadzu Corporation, Japan). MBC was calculated using an extraction efficiency factor (K_{ec}) of 0.25.

3.2.9 Basal respiration

Basal respiration was measured in duplicate on samples collected pre-harvest. The production of CO_2 by metabolizing organisms was measured by placing 20 g (dry weight)

of soil, moistened to 30% water, in a mason jar (500 mL) fitted with a lid and a septum to allow headspace gas sampling. The soil was incubated at 22ºC for 6 weeks. The headspace gas was sampled once a week with a needle attached to a 10 mL syringe and stored in a Vacutainer® until CO_2 analysis. The jars were opened every week after gas sampling to ensure aerobic conditions. The CO_2 produced was determined with a Hewlett Packard 5890 series II Gas Chromatograph equipped with a 10 ft porapak Q column (80/100 19096a-ODO) and attached to a Hewlett Packard 3395 integrator. The initial temperature of the oven was 70ºC for 1.75 min and was programmed to increase at a rate of 52ºC per min to reach 150 ºC for 1.2 min. Helium was used as carrier gas and the flow rate was 50 mL min^{-1}. The average CO_2 production was determined and expressed in μg CO_2-C g^{-1} dry soil h^{-1}.

3.2.10 Microbial community structure

The microbial community structure was determined in 2003 on soil samples collected pre-harvest using FAME analysis. A modification of the method proposed by Schutter and Dick (2000) was used. Approximately 3 g of soil was incubated in 15 mL of 0.2 M KOH in methanol at 37ºC for an hour in a screw-cap glass centrifuge tube. During this step, ester-linked fatty acids were released and methylated. The pH of the tube content was neutralized by adding 3 mL of 1 M acetic acid and the FAMEs were partitioned into an organic phase by adding 15 mL of hexane:chloroform (4:1, v/v). The hexane layer was transferred to a clean glass tube and evaporated under a stream of N_2. In the final step, FAMEs were dissolved in 1 mL of iso-octane containing 24.5 ng/μL of nonadecanoate (C19:0) used as an internal standard.

FAMEs were analysed in split mode (100:1) using a Hewlett Packard 6890 Gas Chromatograph equipped with a 25 m Ultra2 column (cross-linked 5% diphenyl-95% di-methylpolysiloxane; internal diameter, 0.22 mm; film thickness, 0.33 μm; Agilent J&W 19091B-102) and flame ionization detector. The temperature was programmed to increase from 170ºC to 310ºC at a rate of 20ºC per min., and held at 310ºC for 1 min. Detector and inlet temperatures were 300ºC and 250ºC, respectively. Hydrogen was the carrier gas, nitrogen was the make up gas and the flame was supported by air at flow rates of 30, 30 and 400 mL min^{-1}, respectively. We used the same setting as in the Microbial

Identification Systems (MIS) protocol (MIDI, Inc., Newark, Delaware, USA, www.midi-inc.com) (Sasser, 1990; Schutter and Dick, 2000; Buyer, 2002). The retention times of the peaks were converted to equivalent chain length (ECL) values (Sasser, 1990). Peaks were identified based on their retention time (ECLs) as compared with commercial FAME standards (Supelco 37 Component FAME Mix cat.#47885-U; Supelco Bacterial Acid Methyl Esters cat.#47080-U; Matreya PUFA-2 cat.#1081; Matreya Bacterial Acid Methyl Esters CP Mix cat.#1114; Matreya cis-11-Hexadecenoic Acid cat.#1208 and Matreya 10-Methyloctadecanoate cat.#1763). This led to the identification of over 70 FAME. A subset of our samples was sent to a certified external laboratory (Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, QC) where samples were analysed using the Sherlock Microbial Identification System® (MIDI, Inc., Newark, Delaware, USA) and peak identifications were cross-validated.

Fatty acid nomenclature is of the form A:BωC where A designates the total number of carbon atoms, B the number of double bonds, and C the distance of the closest unsaturation from the aliphatic end of the molecule. The suffixes c for *cis* and t for *trans* refer to geometric isomers. The prefixes "i" and "a" refer to iso and anteiso methyl-branching, respectively. Cyclopropane fatty acid is indicated by the prefix Cy, methyl and hydroxy groups are indicated by Me and OH respectively.

A total of 32 fatty acids were quantified to elaborate a FAME profile. Total FAME content was expressed as nanograms FAME per gram dry soil and individual FAME abundance was expressed as a percentage of total FAMEs. FAMEs 14:0, 15:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7c and cy19:0 were considered to represent bacterial FAMEs as indicated by the work of Allison et al. (2005). The sum of these was used to express the percentage of bacterial FAMEs in the soil. Fatty acids 18:1ω9c and 18:2ω6,9c, were considered to represent fungal FAMEs and their sum was used to express the percentage of fungal FAMEs (Madan et al., 2002). Fatty acid 16:1ω5c was considered to represent arbuscular mycorrhizal fungi (AMF) (Madan et al., 2002; Allison et al., 2005). The relative abundance of fungi as compared to bacteria was expressed by the ratio of fungal: bacterial FAME. The ratio (cy17:0+ cy19:0) to their precursors (16:1ω7c + 18:1ω7c) (cyclo/precursor ratio) was determined as an indicator of the status of Gram(-) bacteria. The fatty acids 16:1ω7c and 18:1ω7c are

increasingly converted to the cyclopropyl fatty acids cy17:0 and cy19:0 respectively when Gram(-) bacteria switch from an exponential to a stationary growth phase (Tunlid et al., 1985; Guckert et al., 1986). High cyclo/precursor ratio has been associated with stressful conditions such as carbon and O_2 limitations, high temperature and acidic conditions and might indicate that Gram(-) bacteria are in a stationary rather than logarithmic growth phase (Law et al., 1963; Knivett and Cullen, 1965; Thomas and Batt, 1969; Allison et al., 2005;).

3.2.11 Statistical analyses

Statistics were performed using Statistix®8.0 for Windows. Analysis of variance was used to detect the effect of cropping systems on measured soil variables. Bonferroni's test was used for comparison of means. Correlations between variables were made using Spearman's r^2 coefficient. For all tests of significance, α=0.05.

Principal component analysis (PCA) was used to determine if cropping systems could be discriminated based on their FAME profiles and redundancy analysis (RDA) was used to visualize the relationship between cropping systems, FAMEs and cropping systems characteristics. RDA is a canonical form of PCA in which the axes are restricted to be linear combinations of explanatory variables and help to assess the relationship between the explanatory variables and response variable values (FAMEs). In the resulting ordination diagram, the cropping systems are represented by points, and explanatory and response variables are represented by arrows projecting from the origin. The response variables' arrows point in the direction of maximum variation of the FAMEs and their length is proportional to the rate of change and importance of the considered FAMEs in explaining site differences. Longer explanatory variables' arrows indicate a larger effect of that variable on the total response variables (FAMEs) variation. The cosine of the angle formed by two arrows is an indicator of the correlation between the two variables, a smaller angle indicating a greater correlation.

For the RDA in this study, the FAME groups that showed correlation with soil structure variables and total C were used as response variables (called "species" in ordination models). The explanatory variables were the cropping system characteristics: tillage system (one qualitative variable: tillage), root system morphology (two qualitative

variables: fibrous or tap roots), root presence (two qualitative variables: continuous root presence or temporary root presence), total above-ground biomass production (t.ha^{-1}.year^{-1}), amount of above-ground biomass returned to the soil after harvest (t.ha^{-1}.year^{-1}) and its composition including C:N ratio, % lignin, lignin:N ratio, % fiber and % hemicellulose, estimated below-ground biomass (t.ha^{-1}.year^{-1}) and its composition including C:N ratio, % lignin, lignin:N ratio, % fiber and % hemicellulose. The qualitative explanatory variables were transformed into dummy binary variables. The focus of the ordination was on correlations between FAME groups. The later were centered and standardized.

The Monte Carlo permutation test was performed to evaluate the significance of the relationship between response variables and the whole set of explanatory variables. The test performed 199 unrestricted permutations under the reduced model with block used as a covariable. Monte Carlo test, PCA and RDA were performed using the program CANOCO for Windows (Version 4.0).

3.3 RESULTS AND DISCUSSION

3.3.1 Cropping system characteristics

The amount and quality of the biomass produced by the six cropping systems are presented in Table 3.3. Over three years, C and Sg produced significantly more above-ground biomass (shoot + leaves + grain) than the other cropping systems. The estimated below-ground biomass for Sg was significantly higher than for C, RC and T throughout the study, indicating that more biomass was returned to the field in Sg than in the other cropping systems. Cropping systems S and RC produced similar amounts of above-ground biomass over the three years but more biomass was returned to the field in S since no above-ground biomass was returned to the field in RC.

The above-ground residue and roots of the crops involved in the six cropping systems exhibited different chemical characteristics (Table 3.3). For example, timothy roots are characterized by intermediate C/N ratio, % fiber and % hemicellulose with high lignin and lignin/N ratio while red clover roots, the other perennial crop, are characterized by low C/N, % lignin, lignin/N, % hemicellulose and % fiber. The cropping systems of this study thus contributed differently in terms of quality and quantity of carbon inputs to soil.

3.3.2 Soil structure and carbon content

By the end of the third growing season, analysis of the total soil carbon under the different cropping systems suggests that large carbon inputs to soil in the presence of tillage were insufficient to significantly increase total soil carbon content over three years. Despite their low estimated carbon inputs to soil (Table 3.3), RC and T were the only cropping systems with greater total soil carbon than BF (Table 3.4). Although the estimated carbon input to soil from C and T was large, the carbon content was not different from BF. In these systems as well as in S, changes in total soil carbon after 3 years might have been too small to be detected against the large background of carbon already present in the soil. In perennial systems, such as RC and T, soil carbon accumulation might have been promoted because carbon inputs, especially from rhizodeposition and exudates, were provided over longer periods of time compared to annual cropping systems (Bruce et al., 1999). Even though there was no above-ground biomass returned to the field in RC and T, below-ground biomass might have contributed importantly to carbon input. As compared to annual species where root death occurs mainly during and after flowering, there is a steady turnover of roots during the season for perennial species and thus potential to deposit large amounts of OM into the soil. In addition, mixing fresh plant residues into the soil and the disruption of soil aggregates by tillage promotes organic matter decomposition (Davidson and Ackerman, 1993); absence of soil disturbance in perennial systems could have contributed to the higher levels of carbon (Paustian et al., 1997).

The large amount of corn and sorghum residues returned to the soil over three years and their high C/N and fiber content (Table 3.3) might explain the greater light fraction carbon (LF-C) observed in C and Sg as compared to BF. This soil carbon fraction represents a transitory pool of OM between fresh plant residues and humified soil OM (Gregorich and Ellert, 1993). Its size is a balance between input to the LF pool and loss through decomposition and is thus influenced by amount and quality of carbon input to soil, as well as any other factor affecting decomposition. Smaller input to the LF in S, RC and T contributed to the lower LF in these systems.

The large amount of residue returned to the soil over three years could be responsible for the significantly higher MBC of Sg, which also exhibited higher soil respiration than

38

other cropping systems (Table 3.4). The MBC was similar in C, S, RC and T but soil respiration was higher in RC and T. The higher C/N and fiber content of soybean and corn residues (above and below-ground biomass) as compared to red clover and timothy roots (Table 3.3) and the fact that there was a continuous input of dead roots in RC and T might partly explain the higher soil respiration under the two perennial crops.

Significant improvement in soil structure as compared to BF was observed only in the Sg and T cropping systems. The fibrous root systems of sorghum and timothy together with their high AMF content (2-6 times higher in Sg and T as compared to the other cropping systems; results not shown) and high MBC could explain the high MWD and low percentage of microaggregates in the soils of these systems. It is likely that occasional tillage during the growing season for weed control, and absence of roots were not favourable for building aggregates in the BF system. Greater aggregate stability is usually associated with increased microbial biomass (e.g., Perfect et al., 1990; Drury et al., 1991) and it is likely that the high microbial biomass and soil respiration in Sg and T indicate high microbial activity. Such activity would be associated with a large production of microbial polysaccharides, acting as binding agents between soil particles and also contributing to the building and stabilization of aggregates in Sg and T.

3.3.4 Microbial community structure

After three growing seasons, the total FAME content, which gives an indication of the size of the microbial community, was significantly different between cropping systems (Table 3.5). The highest total FAME content was measured in the Sg system and reflects results for MBC. The microbial community under the evaluated cropping systems also showed structural differences. FAME groups were expressed as a percentage of total FAMEs to test for differences in microbial community composition rather than differences in size of the microbial group considered. When all FAME groups are considered (Table 3.5), C, RC and T, on the one hand, and S and BF on the other, had similar FAME group compositions. Sg was different from BF for all FAME groups and was characterized by high proportions of fungal, AMF, monounsaturated and polyunsaturated FAMEs and low proportions of bacterial, straight chain, branched chain and hydroxyl FAMEs. The relative abundance of fungi as compared to bacteria

39

(fungi/bacteria) was also significantly different between cropping systems. The ratio was highest in Sg and lowest in T and BF. The soils under Sg and T differed by their proportion of polyunsaturated FAMEs, which was higher under Sg. Polyunsaturated fatty acids represent mainly fungi (Zak et al., 1996; Madan et al., 2002) and their proportions were expected to be lower in cropping systems where soil was tilled since tillage is thought to have a detrimental effect on fungal hyphae (Wardle, 1995; Frey et al., 1999).

Principal component analysis (PCA) was used to visualize similarities between cropping systems based on their microbial community structure (Figure 3.2). Correlations between FAME and principal components (results not shown) help identify the FAME which are most responsible for cropping system discrimination (Figure 3.2). The first principal component (PC1) was negatively related to the abundance of fatty acids 18:1ω9c and 18:2ω6,9c, representing fungal FAMEs, fatty acid 16:1ω5c, representing AMF, and fatty acids 16:1ω7c and 18:1ω7c, both representative of Gram(-) bacteria and precursor of fatty acids cy17:0 and cy19:0, respectively. The first principal component was also positively related to the abundance of these two cyclopropane fatty acids together with the abundances of other bacterial FAME, 14:0, 15:0, i15:0, a15:0, i16:0, i17:0, and common fatty acids 18:0 and 16:0. The third principal component (PC3) was negatively related to the abundance of fungal FAMEs 18:1ω9c and 18:2ω6,9c and positively related to that of fatty acids cy17:0 and cy19:0 and their precursor fatty acids 16:1ω7c and 18:1ω7c.

On the PCA diagram presented in Figure 3.2, Sg (2) and BF (6) showed more dissimilarity in FAME profiles, as previously observed with FAME grouping (Table 3.5). Similar to the percentages of FAME groups presented in Table 3.5, BF at the positive end of PC1 is characterised by bacterial dominance as opposed to fungal dominance in Sg at the negative end of PC1. The position of RC (4) and T (5) on the diagram suggests closely related microbial community structures as confirmed by the similar percentages of FAME groups presented in Table 3.5, where only the cyclo/precursor ratio was different when RC and T were compared.

The cyclo/precursor ratio was considered to be an indicator of Gram(-) bacteria physiological status. Thomas and Batt (1969) found higher cyclo/precursor ratios when microorganisms were in starved conditions and the ratio was also found to be higher in

late logarithmic and stationary growth phases (Law et al., 1963). The lower ratio in Sg (Table 3.5) might indicate that Gram(-) bacteria are more active in Sg than in the other cropping systems. However, since basal respiration was also higher in Sg as compared to the other cropping systems (Table 3.4), the low cyclo/precursor ratio in Sg may reflect active growth of the whole microbial community, rather than a specific growth of Gram(-) bacteria. In the PCA diagram, the position of fatty acids cy17:0 and cy19:0 and their precursors 16:1ω7c and 18:1ω7c at the two opposite ends of PC1 (FAMEs not represented in Figure 3.2) might indicate a gradient in the physiological status of Gram(-) bacteria along PC1. Formation of cy17:0 and cy19:0 from their precursors within the plasma membrane of bacteria has been associated with stressful conditions such as C and O_2 limitations, high temperature and acidic conditions, and might indicate that Gram(-) bacteria are in a stationary rather than logarithmic growth phase (e.g., Knivett and Cullen, 1965; Tunlid et al., 1985; Guckert et al., 1986; Allison et al., 2005). Thus the Gram(-) bacteria, and probably the microbial community in general, might be more active in cropping systems found at the negative end and less active in cropping systems at the positive end of PC1.

3.3.5 Correlation between microbial community properties and soil structure and carbon content.

Soil microorganisms undoubtedly play a role in building and maintaining soil structure (Cheshire, 1979; Tisdall and Oades, 1982; Lynch and Bragg, 1985). In this field study, high MWD and greater proportion of large soil aggregates appear to be associated with a larger sized microbial community and increased abundance of most FAME groups (Table 3.6). Microbial biomass carbon and total FAMEs, two correlated measures of the size of the living microbial community ($r^2= 0.69$ at $\alpha < 0.005$), were both highly positively correlated with MWD of dry aggregates and macroaggregates while highly negatively correlated with microaggregates (Table 3.6). Bacterial, AMF, monounsaturated and branched chain fatty acids contents showed similar correlations, suggesting that increased biomass of microorganisms associated with these fatty acids (bacteria, arbuscular mycorrhizal fungi, Gram(-) and Gram(+) bacteria, respectively) would increase the MWD and proportion of soil macroaggregates. Soil basal respiration

(CO_2) was also positively correlated with MWD and macroaggregates. Although not necessarily the case, a larger microbial population may suggest higher microbial activity and more microbial polysaccharides released into the soil as a result of decomposition. The important role these polysaccharides play on soil aggregation may explain why a larger microbial community and greater microbial activity would result in greater soil aggregation.

Correlations were also found between soil carbon content, soil structure variables and proportions of the different FAME groups (Table 3.6) suggesting that the size but also the composition of the microbial community would affect soil structure and carbon content. Higher proportions of AMF FAMEs were associated with higher MWD, increased proportions of macroaggregates and fewer microaggregates, while % fungal FAMEs was only weakly correlated ($0.01 < P < 0.05$) with MWD and not correlated with other soil structural features. The absence of correlation between fungal FAME (% and $ng.g^{-1}$ dry soil) and macroaggregates is surprising since it is widely accepted that plant roots and fungal hyphae play an important role in macroaggregation by physically holding together soil particles and microaggregates. However, some studies have also shown no correlations between fungi and soil aggregation (e.g., Degens et al., 1994; Rillig et al., 2003).

Interestingly, there were no correlations between % bacterial FAME and structural variables but % branched chain fatty acids, associated with Gram(+) bacteria (Zak et al., 1996), were negatively correlated with the % macroaggregates and MWD of dry aggregates while % monounsaturated fatty acids, associated with Gram(-) bacteria (Zak et al., 1996), were positively correlated with the % macroaggregates and MWD of dry aggregates (Table 3.6). This may suggest that Gram(+) and Gram(-) bacteria might not affect aggregation in the same way. Branched chain and monounsaturated fatty acids have also been shown to respond to substrate availability (Thomas and Batt, 1969; Bossio and Scow, 1998; Zelles et al., 1992), their proportions decreasing and increasing, respectively, with increasing substrate availability. On the other hand, the cyclo/precursor ratio has been associated with the growth stage status of Gram(-) bacteria and was shown to increase when the bacteria are in a stationary rather than a logarithmic growth stage (Guckert et al., 1986; Tunlid et al., 1985). In this study, % monounsaturated fatty acids

was positively correlated with MWD, % macroaggregates and negatively correlated with % microaggregates while the cyclo/precursor ratio and % branched chain FAMEs were negatively correlated with MWD and positively correlated with % microaggregates (Table 3.6). These correlations suggest that macroaggregation and MWD might increase when greater quantity of OM and/or OM with better quality (more easily decomposed) are accessible to microorganisms. Increased substrate availability may lead to higher microbial activity with higher production of microbial aggregating agents and could explain increased MWD and macroaggregation with increased organic carbon availability. Since monounsaturated and branched chain fatty acids are also Gram(-) and Gram(+) bacteria markers, respectively (Zak et al., 1996; Zelles, 1997), we may hypothesize that greater MWD and % macroaggregates are associated with greater carbon availability to Gram(-) bacteria. This hypothesis is supported by experiments where Gram(-) bacteria were suggested to feed preferentially on freshly added organic material (Waldrop and Firestone, 2004; Kramer and Gleixner, 2006).

MBC, total FAME and most FAMEs groups (ng.g^{-1} soil) were positively correlated with total organic carbon content of soil (Table 3.6). Correlations between soil carbon content and indicators of carbon availability to microorganisms (% branched chain and % monounsaturated FAME) as well as with indicators of microbial activity (CO_2-C and cyclo/precursor) suggest that high total soil C might be achieved when a large microbial community feeds on highly available substrates. It is interesting that greater CO_2 production resulted in more soil carbon. During microbial decomposition, carbon is lost as CO_2 but some is also incorporated into the soil microbial biomass and SOM. Thus, greater soil respiration might be associated with more carbon incorporated into these soil carbon fractions. Greater soil respiration might also be associated with greater production and release of microbial polysaccharides in the soil, which in turn might increase aggregation, thus protecting soil carbon.

3.3.6 Relationship between microbial community structure and cropping system characteristics

The Monte Carlo permutation test demonstrated that the relation between the 18 cropping system characteristics and the 12 FAME groups was strong (P=0.005 for both

the first and all canonical axes). Figure 3.3 shows the cropping system points, the cropping system characteristics variables and the FAME arrows for the RDA ordination. As determined by forward selection and the Monte Carlo permutation test, the ranking of explanatory variables showed that below-ground biomass ($P<0.005$) and root lignin content ($P<0.005$) were the cropping system characteristics with the greatest influence on FAME group sizes and proportions. These two variables explained 90% of the total variance explained by all the explanatory variables; the remaining 10% being attributed to tillage practice, root morphology and % fiber, the above-ground biomass production and % hemicellulose, as well as total above-ground biomass returned to the soil.

For the RDA analysis, the above-ground biomass quality variables were not included for RC, T and BF since above-ground residues were not returned to the field after harvest. In C, Sg and S, above-ground residue had been incorporated into the soil nearly one year before soil sampling. It is likely that one year later, the impact of above-ground residue incorporation on microbial community size and structure are not as important as the effects of the root system, where rhizodeposition and root senescence occur throughout the growing season, especially under the perennial crops. Below-ground biomass and fibrous roots of grass species were highly and positively correlated with total FAMEs ($ng.g^{-1}$ soil), monounsaturated fatty acids (% and $ng.g^{-1}$ soil), AMF fatty acids (% and $ng.g^{-1}$ soil), bacterial and branched fatty acids ($ng.g^{-1}$ soil). Negative correlations were observed with cyclo/precursor ratio, percentage of branched fatty acids and bacteria. If equal root biomass is considered, the root surface area in contact with the soil is greater with a fine fibrous root system than a coarse fibrous root system or a tap root and, consequently, the effect of the root system on the soil microbial community should be greater. The correlations found here indicate the importance of root biomass as a carbon input to soil, but also the importance of root morphology in influencing the size (total FAMEs and FAME groups in $ng.g^{-1}$ soil) and the composition of the microbial community (% FAME groups). As an extension, rhizodeposition and root material availability to the microbial community might be greater in a fibrous root system due to greater surface area contact between roots and microorganisms, and this could explain the correlation obtained with cyclo/precursor ratio, branched and monounsaturated fatty acids. These fatty acids have been used as indicators of nutritional stress, the proportion of

monounsaturated fatty acids increasing, and the cyclo/precursor ratio and the proportion of branched fatty acids decreasing with increasing substrate availability (Thomas and Batt, 1969; Bossio and Scow, 1998; Zelles et al., 1992; Guckert et al., 1986; Tunlid et al., 1985). In no-till perennial cropping systems like RC and T where carbon inputs were essentially from the root system, the roots' morphology would greatly influence its impact on the microbial community since there was no tillage to mix the soil and increase the contact between roots and microbes.

Below-ground biomass, above-ground biomass production and amount returned to the soil may have an effect on the size of the microbial community since the amount of microbial biomass is directly related to the quantity of carbon available for microbial growth (Anderson and Domsch, 1989; Wardle, 1992). Root lignin and fiber on the other hand may influence the structure and the size of the microbial community because these are more complex compounds that only a few organisms are able to decompose efficiently.

Of the eight variables influencing FAME groups, the three most important accounted for 92.4% of the total variance explained by all the explanatory variables and were related to the root system. Except for above-ground hemicellulose, the Monte Carlo permutation test found no effect of above-ground biomass quality variables on variation among FAME groups. Part of this might be due to the timing of soil sampling. Indeed, in the third growing season, soil was sampled before residue incorporation. The last above-ground crop residue incorporation to soil had taken place almost one year previously, and at this time, the initial chemical characteristics of incorporated above-ground residues might no longer have been affecting the composition of the microbial community. The longer residence time in soil of root than shoot derived carbon (Bottner, 1982; Gale and Cambardella, 2000) might also be responsible for the greater influence of plant roots on the microbial community.

3.4 CONCLUSION

After three years of cultivation, the six cropping systems had altered the levels of soil variables often used as indicators of carbon accumulation in soil. The greatest differences were observed between the sorghum-based cropping system and the bare fallow. Soil carbon content and aggregate size distribution were related to the composition of the microbial community. The latter was mainly influenced by root biomass, chemical composition and morphology.

This study showed that it takes much more than increasing carbon inputs to soil for carbon to accumulate. This was illustrated by the fact that although untilled perennial cropping systems contributed to soil carbon exclusively through their root systems, they increased soil carbon more than the highly productive annual cropping system involving corn, that had estimated total biomass inputs to soil nearly three times greater than for perennial systems. Absence of soil disturbance by tillage and extended period of active growth in perennial systems likely contributed to the higher soil carbon in these systems.

Correlations found between soil structure variables and FAME groups associated with different microorganisms suggest that the composition of the microbial community might be important at building soil aggregates. Interestingly, correlations obtained between soil structure variables and bacterial FAME groups suggested that Gram(-) bacteria might be involved in soil macroaggregation whereas Gram(+) bacteria do not. Increased soil carbon content was associated with increased microbial activity pointing to the fact that even though decomposition is a process by which organic carbon is lost from soil into the atmosphere, minimizing OM decomposition might not be the best strategy when trying to increase soil carbon. Indeed, beside the fact that this process is necessary to maintain soil fertility and make nutrients available for plant growth (thus contributing to higher crop yield), it also contributes to OM stabilization and storage in soil by improvement of soil structure (through the production of binding agents by the microbial community) and transformation of OM into more resistant and stable compounds.

Greater accumulation of soil carbon in the perennial systems where above-ground biomass was not returned to the field emphasizes the importance of the root system for carbon storage. There is a large amount of data available on the effect of crops and management on soil structure and C content; however, few studies emphasize the effect

of below-ground contributions. As opposed to above-ground crop materials, there is little available data on root material, exudate and rhizodeposition decomposition, despite the obvious importance of their contribution to soil carbon. More research effort on the hidden part of crops is needed to fully understand the mechanisms of carbon storage in soil.

Table 3.1: Cropping systems characteristics in terms of crop, tillage practice, root morphology and source of carbon inputs to soil.

Cropping system	Crop name	Type of crop	Variety	Tillage	Roots morphology†	Roots activity	Carbon inputs
C	corn	annual	Pioneer 3921	surface	coarse fibrous	planting to harvest	rhizodepositions, roots, above-ground residue
Sg	sorghum	annual	Sudangrass hybrid	surface	Finer and more fibrous than corn	planting to harvest	rhizodepositions, roots, above-ground residue
S	soybean	annual	OAC Bayfield	surface	not pronounced taproot	planting to harvest	rhizodepositions, roots, above-ground residue
RC	red clover	perennial	Khun	None	pronounced taproot	continuous	rhizodepositions, roots
T	timothy	perennial	Champ	None	shallow fine fibrous	continuous	rhizodepositions, roots
BF	none	none	none	surface	none	none	none

† Weaver, 1926.

Table 3.2: Dates of field operations

Operation	Crop	2001	2002	2003
Planting	corn	May 31	May 31	May 20
	sorghum	May 31	May 31	May 20
	soybean	May 31	May 31	May 20
	red clover	May 31	NA	NA
	timothy	May 31	NA	NA
First NH_3NO_4 fertilizer application	corn	May 31	June 7	May 27
	sorghum	May 31	June 7	May 27
	timothy	May 31	May 27	May 20
Second NH_3NO_4 fertilizer application	corn	July 6	July 7	July 2
	sorghum	July 6	June28	July 2
First cut	red clover	October 16	June 25	June 16
	timothy	not cut	June 25	June 16
Second cut	red clover	not cut	August 21	August 5
	timothy	not cut	not cut	August 27
Harvest	corn	October 16	October 11	October 14
	sorghum	October 16	October 21	October 14
	soybean	October 2	September 18	September 24
Soil sampling		May 22	May 23	May 5
		July 18	September 18	June 17
		October 1		August 5
				September 18

NA : not applicable

Table 3.3: Above and below-ground biomass production (3 years total) and selected chemical characteristics of roots and above-ground residue in each cropping system

Cropping system	Above-ground							Roots					
	Biomass produced (t ha^{-1})†	Biomass returned to the field (t ha^{-1})†	C/N ‡	Lignin ‡ (%)	Lignin/N ‡	Fiber ‡ (%)	Hemi-cellulose (%)	Estimated below-ground biomass (t ha^{-1})†	C/N	Lignin (%)	Lignin/N	Fiber (%)	Hemi-cellulose (%)
C	50a	29b	72	6	11	81	33	11b	52	11	10	77	31
Sg	46a	46a	65	6	8	62	27	27a	ND	ND	ND	ND	ND
S	17b	11c	48	10	11	63	16	4c	34	20	16	80	17
RC	18b	0d	17	15	7	47	10	10b	14	13	5	43	13
T	13*	0d	20	6	3	50	21	6*	26	29	24	68	20
BF	ND	ND	NA	NA	NA	NA	NA	ND	NA	NA	NA	NA	NA

Numbers within a column followed by the same letter are not significantly different at the 0.05 probability level. The cropping systems C, Sg, S, RC, T and BF refer to corn, sorghum, soybean, red clover, timothy and bare fallow respectively.
†:Total biomass for three years; *:Total biomass for two years (2002 and 2003); ‡ : Chemical characteristics for above-ground biomass do not include grain except for sorghum where all above-ground biomass produced was returned to the soil; C/N: carbon-to-nitrogen ratio; Lignin/N: lignin-to-nitrogen ratio; ND: not determined; NA: not applicable.

Table 3.4: Total carbon, light fraction carbon (LF-C), microbial biomass carbon (MBC), basal soil respiration (CO_2-C) and effect of cropping system on mean weight diameter of dry aggregates (MWD) and proportion of microaggregates after three growing seasons.

Cropping system	Total C (g.kg^{-1})	LF-C (g.kg^{-1})	MBC (ug.g^{-1})	CO_2-C (ng.g^{-1}.h^{-1})	MWD (mm)	% Micro aggregates
C	12.1c	1.1a	282bc	297c	0.86bc	46ab
Sg	14.4ab	1.1a	471a	689a	1.21a	33c
S	13.8abc	0.6ab	283bc	290c	0.82bc	50a
RC	14.9a	0.9ab	327bc	417b	0.89bc	42abc
T	15.2a	0.9ab	347b	498b	1.05ab	35bc
BF	12.7bc	0.5b	242c	268c	0.72c	49a

Numbers within a column followed by the same letter(s) are not significantly different at the 0.05 probability level. The cropping systems C, Sg, S, RC, T and BF refer to corn, sorghum, soybean, red clover, timothy and bare fallow respectively.

Table 3.5: Relative amount (%) of fatty acids methyl ester (FAME) groups in the soil under the different cropping systems after three growing seasons

	C	Sg	S	RC	T	BF
Total FAME (μg g^{-1} dry soil)	54bc	87a	51bc	59b	68b	37c
Bacterial FAME (% total FAME)	31ab	28b	33ab	32ab	33ab	35a
Fungal FAME (% total FAME)	18b	22a	14bc	14c	12c	13c
AMF FAME (% total FAME)	8ab	10a	6ab	8ab	11a	4b
Straight chain (% total FAME)	29ab	26b	31ab	29ab	30ab	32a
Branched chain (% total FAME)	19abc	16c	21ab	20ab	18bc	22a
Monounsaturated (% total FAME)	31b	38a	29bc	30b	34ab	24c
Polyunsaturated (% total FAME)	6ab	9a	5b	5b	5b	5b
Hydroxyl (% total FAME)	11ab	7c	11ab	10abc	9bc	13a
Fungal FAME / bacterial FAME	0.56b	0.77a	0.41bc	0.41bc	0.37c	0.38c
Cyclo/precursor	0.41ab	0.27c	0.45a	0.45a	0.31bc	0.52a

Numbers within a row followed by the same letter(s) are not significantly different at the 0.05 probability level. The cropping systems C, Sg, S, RC, T and BF refer to corn, sorghum, soybean, red clover, timothy and bare fallow respectively. AMF: arbuscular mycorrhizal fungi.

Table 3.6: Spearman's correlation coefficients (r^2 value) between total carbon (Total C), mean weight diameter of dry aggregates (MWD), percentage of macro and microaggregates and microbial community properties (n=24).

	MWD	Macro aggregates	Micro aggregates	Total C
MBC ($\mu g.g^{-1}$ soil)	0.57***	0.40***	0.53***†	0.40***
Total FAME ($ng.g^{-1}$ soil)	0.46***	0.32***	0.45***†	0.29**
Fungal FAME ($ng.g^{-1}$ soil)	0.37***	ns	0.28**†	ns
Fungal FAME (% total FAME)	0.18*	ns	ns	ns
AMF FAME ($ng.g^{-1}$ soil)	0.39***	0.30**	0.42***†	0.30**
AMF FAME (% total FAME)	0.21*	0.19*	0.27*†	0.25*
Bacterial FAME ($ng.g^{-1}$ soil)	0.50***	0.41***	0.51***†	0.48***
Bacterial FAME (% total FAME)	ns	ns	ns	ns
Branched chain ($ng.g^{-1}$ soil)	0.46***	0.37***	0.44***†	0.51***
Branched chain (% total FAME)	0.24*†	ns	0.30**	ns
Monounsaturated ($ng.g^{-1}$ soil)	0.47***	0.32***	0.46***†	0.27*
Monounsaturated (% total FAME)	0.41***	0.27*	0.46***†	0.25*
cyclo/precursor	0.40***†	0.30**†	0.45***	0.22*†
CO_2-C ($ng.g^{-1}$ $soil.h^{-1}$)	0.51***	0.46***	0.57***†	0.42***

*: $0.01 < P < 0.05$; **: $0.005 < P < 0.01$; ***: $P < 0.005$; ns: not significant
†: negative correlation; FAME : fatty acids methyl esters; CO_2-C: soil basal respiration; MBC: microbial biomass carbon

Block 4	S	T	RC	BF	Sg	C
Block 3	T	C	Sg	RC	S	BF
Block 2	Sg	BF	RC	C	T	S
Block 1	C	Sg	BF	S	RC	T

Figure 3.1: Layout of site. The cropping systems C, Sg, S, RC, T and BF refer to corn, sorghum, soybean, red clover, timothy and bare fallow respectively.
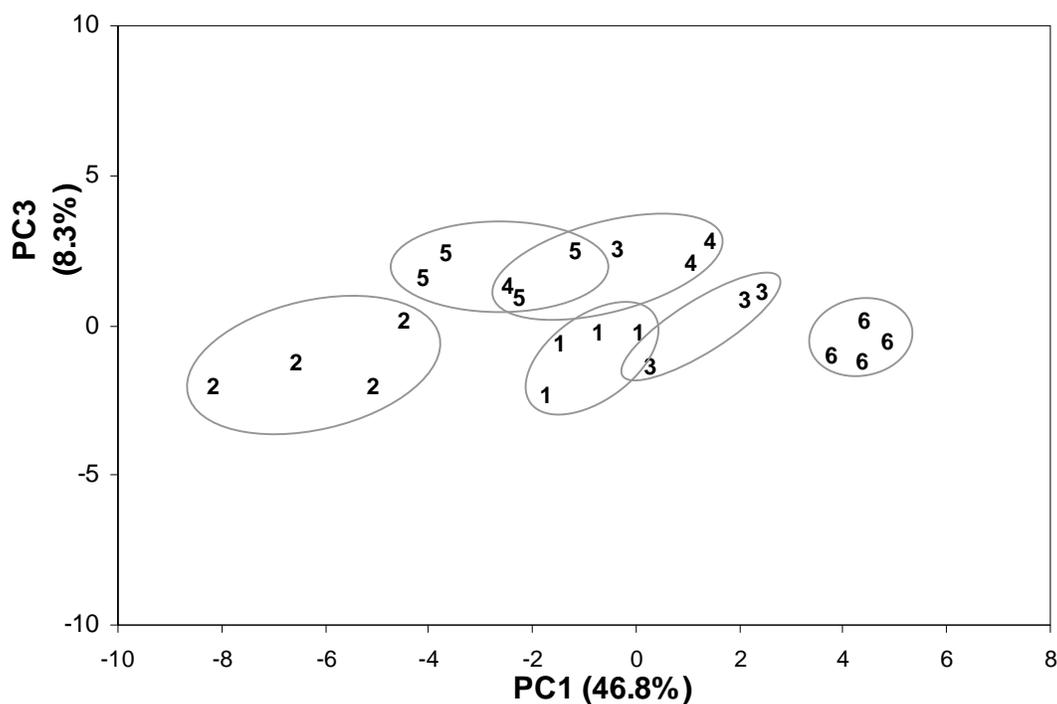
Figure 3.2: Two dimensional plot of the six cropping systems on the first and third principal components (PC1 and PC3) after principal component analysis of their fatty acid profiles. PCA was conducted with 32 fatty acids (percentage of total FAME) that were present in all the samples. 1: corn, 2: sorghum, 3: soybean, 4: red clover, 5: timothy, and 6: bare fallow.
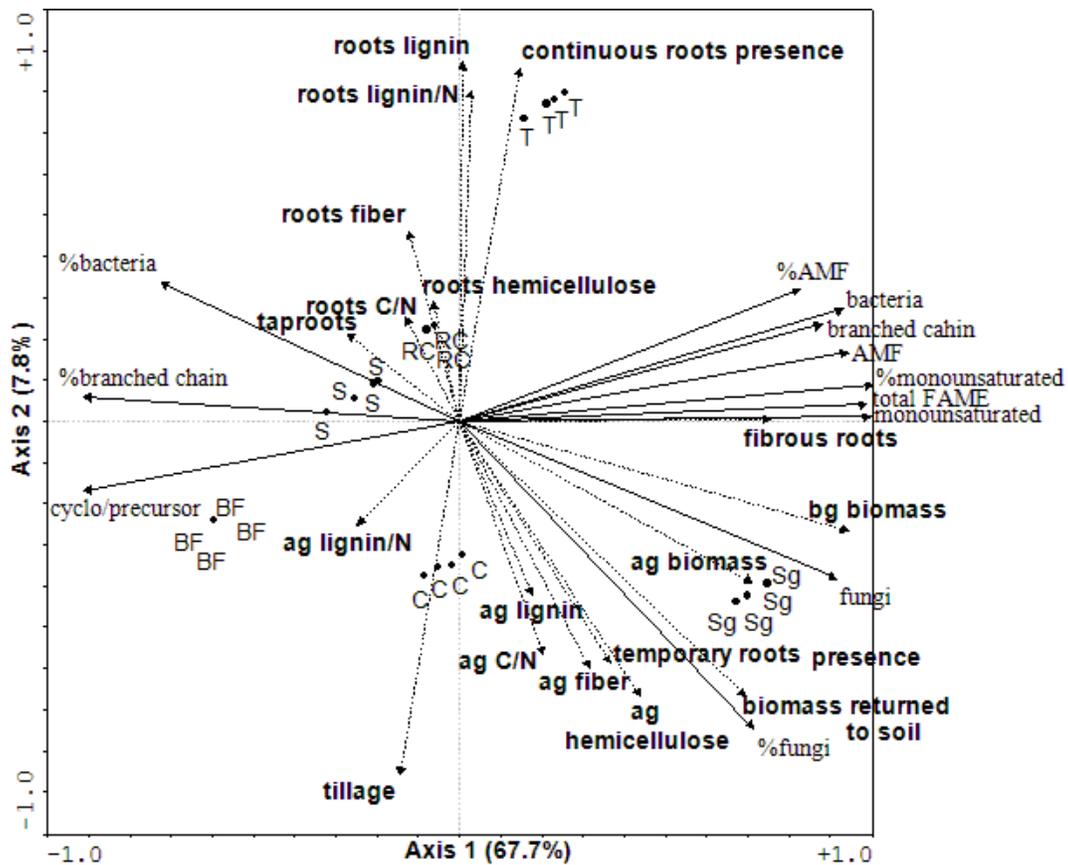
Figure 3.3: Redundancy analysis (RDA) biplot of FAME data for the different cropping systems, using 12 FAME groups as species and 18 explanatory variables. The cropping systems C, Sg, S, RC, T and BF refer to corn, sorghum, soybean, red clover, timothy and bare fallow respectively. ag: above-ground; bg: below-ground.

REFERENCES

Allison, V. J., Miller, R.M., Jastrow, J. D., Matamala, R., and. Zak, D. R. 2005. Changes in Soil Microbial Community Structure in a Tallgrass Prairie Chronosequence. Soil Science Society of America Journal 69:1412–1421

Anderson, T.H., and K.H. Domsch. 1989. Ratio of microbial biomass carbon to total biomass organic carbon in arable soils. Soil Biology an Biochemistry 21:471–479.

Angers, D.A., Edwards, M.L., Sanderson, J.B. and Bissonnette, N. 1999. Soil organic matter quality and aggregate stability under eight potato cropping sequences in a fine sandy loam of Prince Edward Island. Canadian Journal of Soil Science 79: 411-417

Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C. 1999. Seasonal changes in microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry 31: 1021-1030.

Beare, M.H., Cabrera, M.L., Hendrix, P.F. and Coleman, D.C. 1994. Aggregate-protected and unprotected pools of organic matter in conventional and no-tillage soils. Soil Science Society of America Journal 58: 787-795.

Beare, M.H., Hu, S., Coleman, D.C. and Hendrix, P.F. 1997. Influences of mycelial fungi on soil aggregation and organic matter storage in conventional and no-tillage soils. Applied Soil Ecology 5: 211-219.

Bélanger, G., Brégard, A. and Michaud, R. 2002. Phosphorous uptake and concentration of timothy genotypes under varying N applications. Crop Science 42:2044–2048.

Bissonnette, N., Angers, D.A., Simard, R.R. and Lafond, J. 2001. Interactive effects of management practices on water-stable aggregation and organic matter of a Humic Gleysol. Canadian Journal of Soil Science 81: 545–551.

Bossio, D.A. and Scow, K.M. 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35:265-278.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R. and Paustian, K. 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16: 195-208.

Bottner, P. 1982. Biodegradation du material vegetal en milieu herbace. Acta Oecologia 3: 155-182.

Brégard, A., Bélanger, G., Michaud, R. and Tremblay, G.F. 2001. Biomass partitioning, forage nutritive value and yield of contrasting genotypes of timothy. Crop Science 41:1212–1219

Bruce, J.P., Frome, M., Haites, E., Janzen, H., Lal, R. and Paustian K. 1999. Carbon sequestration in soils. Journal of Soil and Water Conservation, first quarter: 382-389

Buyanovsky, G.A. and Wagner, G.H. 1987. Carbon transfer in a winter wheat (Triticum aestivum) ecosystem. Biology and Fertility of Soils 5:76-82.

Buyanovsky, G.A. and Wagner, G.H. 1997. Crop residue input to soil organic matter in the Sandborn field. p73-83 *In* E.A Paul et al. (ed) Soil Organic Matter in Temperate Agroecosystems. CRC Press, Boca Raton, FL.

Buyer, J. 2002. Identification of bacteria from single colonies by fatty acid analysis. Journal of Microbiological Methods 48:259-265.

Calderon, F.J., Jackson, L.E., Schow, K.M., Rolston, D.E. 2001. Short-term dynamics of nitrogen, microbial activity and phospholipids fatty acids after tillage. Soil Science Society of America Journal 65:118-126.

Campbell, M.C., Bennett, S.J. and Cocks, P.S. 1998. Above and below ground: A comparative study of the root and shoot growth of pasture legumes. Proceedings of the 9th Australian Agronomy Conference, Australian Society of Agronomy.

Carter, M.R. 1996. Analysis of soil organic matter storage in agroecosystems. In: Advances in soil sciences: Structure and Organic Matter Storage in Agricultural Soils. Edited by M.R. Carter and B.A. Stewart. CRC Press Inc.

Chantigny, M.H., Angers, D.A., Prevost, D., Vezina, L.P. and Chalifour, F.P. 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Science Society of America Journal 61: 262-267.

Cheshire, M.V. 1979. Nature and origin of carbohydrates in soils. Academic Press, London.

Davidson, E.A. and Ackerman, I.L. 1993. Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry 20: 161–193

Degens, B.P., Sparling, G.P. and Abbott, L.K. 1994. The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long term clover-based and grass pastures. European Journal of Soil Science 45: 459-468.

Drijber, R.A., Doran, J.W., Parkhurst, A.M. and Lyon, D.J. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology and Biochemistry 32:1419-1430.

Drury, C.F., Stone, J.A. and Findlay, W.I. 1991. Microbial biomass and soil structure associated with corn, grasses, and legumes. Soil Science Society of America Journal 55: 805-811.

Duiker, S.W. and Lal, R. 1999. Crop residue and tillage effects on carbon sequestration in a Luvisol in central Ohio. Soil and Tillage Research 52: 73-81.

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

Eneji, E., Inanaga, S., Muranaka, S., Li, J., An, P., Hattori, T. and Tsuji, W. 2005. Effect of calcium silicate on growth and dry matter yield of *Chloris gayana* and *Sorghum sudanense* under two soil water regimes. Grass and Forage Science 60: 393-398.

Feeney, D.S., Daniell, T., Hallett, P.D., Illian, J., Ritz, K. and Young, I.M. 2004. Does the presence of glomalin relate to reduced water infiltration through hydrophobicity? Canadian Journal of Soil Science 84: 365–372.

Frey, S.D., Elliott, E.T. and Paustian, K. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology and Biochemistry 31: 573-585.

Gale, W.J. and Cambardella, C.A. 2000. Carbon dynamics of surface residue and root-derived organic matter under simulated no-till. Soil Science Society of America Journal 64:190-195.

Gregorich, E.G. and Ellert, B.H. 1993. Light fraction and macroorganic matter in mineral soils. In: Soil sampling and methods of analysis. Edited by M.R. Carter. Lewis Publishers

Gregorich, E.G., Drury, C.F. and Baldock, J.A. 2001. Changes in soil carbon under long-term maize in monoculture and legume-based rotation. Canadian Journal of Soil Science 81: 21-31.

Huggins, D.R. and Fuchs, D.J. 1997. Long-term N management effects on corn yield a soil C of an Aquic Haplustoll in Minnesota. In: Paul EA et al (eds) Soil organic matter in

temperate agroecosystems: long-term experiments in North America. CRC Press Inc., New York, pp 121–128

Huggins, D.R., Buyanovsky, G.A., Wagner, G.H., Brown, J.R., Darmody, R.G., Peck, T.R., Lesoing, G.W., Vanotti, M.B. and Bundy, L.G. 1998. Soil organic C in the tallgrass prairie-derived region of the corn belt: effects of long-term crop management. Soil and Tillage Research 47:219–234

Ibekwe, A.M. and Kennedy, A.C. 1999. Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant and Soil 206:151-161

Kemper, W.D. and Rosenau, R.C. 1986. Pages 425-442 in A. Klute and A. L. Page, Eds. Aggregate stability and size distribution. Methods of soil analysis. Part 1. Physical and mineralogical methods. Agronomy No 9, 2nd ed. Soil Science Society of America, American Society of Agronomy, Madison, WI.

Klug, M.J. and Tiedje, J.M. 1993. Response of microbial communities to changing environmental conditions: chemical and physiological approaches. *In* Trends in Microbial Ecology. Eds. R Guerrero and C Pedros-Alio (eds). Pp 371-374. Spanish Society for Microbiology, Bacelona, Spain.

Knivett, V.A. and Cullen, J. 1965. Some factors affecting Cyclopropane acid formation in *Escherichia coli.* Biochemistry Journal 96:771–776

Kramer, C. and Gleixner, G. 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. Soil Biology and Biochemistry 38: 3267-3278.

Kunitsky, C., Ostherout, G. and Sasser, M. 2006. Identification of microorganisms using fatty acid methyl ester (FAME) analysis and the MIDI Sherlock Microbial Identification System. *In* Encyclopedia of Rapid Microbiological Methods, Volume III. M.J. Miller (Ed.) DHI Publications, Washington, DC. pp 1-17

Kurdali, F. 2004. Estimates of dry matter yield and nitrogen uptake in sorghum grown on saline and non-saline soils manured with dhaincha plant residues. Journal of Plant Nutrition 27:1611-1633

Larson, W.E., Clapp, C.E., Pierre, W.H. and Morachan, Y.B. 1972. Effects of increasing amounts of organic residues on continuous corn. II. Organic C, N, P and S. Agronomy Journal 64: 204-208.

Law, J.H., Zalkin, H., Kaneshiro, T. 1963. Transmethylation reactions in bacterial lipids. Biochimica and Biophysica Acta 70:143–151

Lynch, J.M. and Bragg, E. 1985. Microorganisms and soil aggregate stability. Advances in Soil Science 2: 134-170

Madan, R., Pankhurst, C., Hawke, B. and Smith, S. 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. Soil Biology and Biochemistry 34:125-128.

Nichols, K.A. and Wright, S.F. 2005. Comparison of glomalin and humic acid in eight native U.S. soils. Soil Science 170: 985–997

Oades, J.M. 1984. Soil organic matter and structural stability mechanisms and implications for management. Plant and Soil 76: 319-337

Oades, J. M. and Waters, A.G. 1991. Aggregate hierarchy in soils. Australian Journal of Soil Research 29: 815-828.

Pankhurst, C.E. 1997. Biodiversity of soil organisms as an indicator of soil health. *In* Biological indicators of soil health. Eds. C.E. Pankhurst, B.M. Doube and V.V. Gupta. Pp 297-324. New York, NY.

Paustian, K., Andren, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., Van Noordwijk, M. and Woomer, P.L. 1997. Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use and Management 13: 230-244.

Perfect, E., Kay, B.D., Van Loon, W.K.P., Sheard, R.W. and Pojasok, T. 1990. Factors influencing soil structure stability within a growing season. Soil Science Society of America Journal, 54: 173-179.

Pikul, J.L. and Allmaras, R.R. 1986. Physical and chemical properties of a Haploxerol after fifty years of residue management. Soil Science Society of America Journal 50: 214-219.

Power, J.F., Koerner, P.T., Doran, J.W. and Wilhelm, W.W. 1998. Residual effects of crop residues on grain production and selected soil properties. Soil Science Society of America Journal 62:1393-1397.

Puget, P., Angers, D.A. and Chenu, C. 1999. Nature of carbohydrates associated with water-stable aggregates of cultivated soils. Soil Biology and Biochemistry 31: 55-63.

Rasmussen, P.E., Allmaras, R.R., Rohde, C.R, and Roager, N.C., Jr. 1980. Crop residue influences on soil carbon and nitrogen in a wheat-fallow system. Soil Science Society of America Journal 44: 596-600.

Reicosky, D.C., Evans, S.D., Cambardella, C.A., Armaras, R.R., Wilts, A.R. and Huggins, D.R. 2002. Continuous corn with mouldboard tillage: residue and fertility effects on soil carbon. Journal of Soil and Water Conservation 57:277–284

Rillig, M.C., Maestre, F.T. and Lamit, L.J. 2003. Microsite differences in fungal hyphal length, glomalin, and soil aggregate stability in semiarid Mediterrenean steppes. Soil Biology and Biochemistry 35: 1257-1260.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Tech. Note #101. Microbial ID, Newark, USA.

Schutter, M.E. and Dick, R.P. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64:1659-1668.

Thomas T.D. and Batt, R.D. 1969. Degradation of cell constituents by starved *Streptococcus lactis* in relation to survival. Journal of General Microbiology 58:347–362.

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

Tisdall, J.M., 1994. Possible role of soil microorganisms in aggregation in soils. Plant and Soil 159: 115-121.

Tunlid, A., Baird, B.H., Trexler, M.B., Olsson, S., Findlay, R.H., Odham, G. and White, D.C. 1985. Determination of phospholipids ester-linked fatty acids and poly beta hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* (L). Canadian Journal of Microbiology 31:1113-1119.

Voroney, R.P., Winter, J.P. and Beyaert, J.P. 1993. *In* Soil sampling and methods of analysis. M.R. Carter (ed.) Lewis Publishers, CRC Press Inc., Boca Raton, FL.

Waldrop, M.P. and Firestone, M.K. 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138: 275–284.

Wardle, D.A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67: 321-358.

Wardle, D.A., 1995. Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26: 105–185.

Weaver, J. E. 1926. Root development of field crops. McGraw-Hill Book Company, Inc. New York.

West, T.O. and Post, W.M. 2002. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. Soil Science Society of America Journal 66:1930-1946.

White, D.C., Stair, J.O. and Ringelberg, D.B. 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. Journal of Industrial Microbiology 17:185-196.

Wilts, A.R., Reicosky, D.C., Allmaras, R.R., and Clapp, C.E. 2004. Long-term corn residue effects: Harvest alternatives, soil carbon turnover and root derived carbon. Soil Science Society of America Journal 68:1342-1351.

Wright, S.F., Starr, J.L and Paltineanu, I.C. 1999. Changes in aggregate stability and concentration of glomalin during tillage management transition. Soil Science Society of America Journal 63:1825-1829.

Zak, D.R. Ringelberg, D.B., Pregitzer, K.S., Randlett, D.L., White, D.C. and Curtis, P.S. 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecological Applications 6: 257-262.

Zelles, L., Bai, Q.Y., Beck, T. and Beese, F. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbiological biomass and community structure in agricultural soils. Soil Biology and Biochemistry 24: 317-323.

Zelles, L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35: 275-294.

Connecting paragraph

Chapter 3 presented the results of a multi-cropping system field experiment designed to study the effect of different cropping systems on soil structure, carbon content and microbial community size, activity and structure. The results suggested a relationship between soil structure and microbial community structure. Greater proportions of bacteria, arbuscular mycorrhizal fungi and Gram(-) bacteria were associated with increased proportions of soil macroaggregates and higher mean weight diameter (MWD) of aggregates; increased proportions of fungi was associated with higher MWD of aggregates. Root biomass and lignin content had the greatest infleunce on size and proportion of microbial groups correlated with soil structure and carbon content. The experiment presented in Chapters 4 and 5 was designed to isolate the impact of plant material chemical characteristics on the structure of the microbial community and to further explore the effect of microbial community structure on soil aggregation. Chapter 4 presents the results of a 196 days incubation experiment where the relationship between soil structure and soil microbial community composition was investigated in order to determine the soil microbial groups the most important at influencing soil structure.

Chapter 4

Relationships between microbial community composition and soil structure during plant material decomposition.

4.1 INTRODUCTION

Soil microorganisms are involved in many soil processes including the formation and stabilization of soil aggregates. Fungal and bacterial polysaccharides, produced and released in the soil mainly during microbial metabolism and organic matter decomposition, affect soil aggregation through their chemical interactions with soil particles (Cheshire, 1979; Oades, 1984). Fungi and bacteria seem to exert their effect on aggregation at different level. It has been postulated that microaggregates are mainly influenced by bacteria and their by-products (Lynch and Bragg, 1985; Tisdall, 1994) while fungi initiate macroaggregate formation with their filamentous hyphae holding together soil particles (Tisdall and Oades, 1982; Oades and Waters, 1991). In a study conducted by Bossuyt et al. (2001) no effect on macroaggregate formation was found by suppressing bacterial growth while suppression of fungal growth significantly reduced macroaggregation. Improvement in soil structure is usually associated with increased microbial biomass (e.g., Perfect et al., 1990; Drury et al., 1991) and macroaggregation has been positively related to length of fungal hyphae (e.g., Degens et al., 1996). However, occasional failures in attempts to relate soil structure with microbial community size (e.g., Angers et al., 1992; Carter et al., 1994; Chantigny et al., 1997) and hyphal length (e.g., Degens et al., 1994) raise additional questions regarding the link between microbial community and their influence on soil structure. Variability in the ability of microorganisms to produce extracellular polysaccharides as well as the diversity in structure of the polysaccharides produced (Sutherland, 1985; Hebbar et al., 1992) suggest that the amount of microbial products released into the soil might not be related to the size of the microbial community and/or that the "binding power" of microbial polysaccharides might vary with the composition of the microbial community. Soil microorganisms also exhibit a variety of cell wall chemical characteristics that may influence their interaction with soil particles and affect aggregation. Thus, the chemical characteristics of microbial cells and metabolites as well as

their effect on the form of organic matter degradation products might affect soil aggregate strength. Since these might be a function of the structural composition of the microbial community, soil structural stability might be related to the composition of the microbial community.

Soil microbial fatty acids have been used to study microbial community composition and were shown to respond to changes in soil environmental conditions, nitrogen fertilisation, tillage practices and plant species (e.g., Bossio and Scow, 1995; Bardgett et al., 1999; Ibekwe and Kennedy, 1999; Calderon et al 2001; Allison et al., 2005). The fatty acid profile of soil provides information on the microbial community structure as some fatty acids are indicators for either fungi, bacteria or actinomycetes (Zak et al., 1996; White et al., 1997; Zelles 1997; Ritchie et al., 2000; Madan et al., 2002).

The objective of this study was to define the relationships between microbial community composition and soil structure with an incubation experiment where a soil was amended with plant materials of different chemical characteristics. We predict soil MWD and proportion of macroaggregates to increase following addition of organic material to soil. We expect greater proportion of fungi to be associated with higher proportion of soil macroaggregates and we aim to identify the importance of different groups of microorganisms (Gram(-) and Gram(+) bacteria, actinomycetes, fungi) at influencing soil structure.

4.2 MATERIALS AND METHODS

4.2.1 Soil

The soil was collected from the surface layer (0-20 cm) of a field located at Ste-Anne-de-Bellevue, Quebec (45°25' N, 73°57' W) one year prior to the experiment and stored moist in a bucket at room temperature. It was a Chateauguay clay loam with 43.4% sand, 22.6% clay and 34% silt and a pH of 7.8. The total organic carbon and nitrogen content were 4 and 0.3% respectively for a C/N ratio of 13. The soil was sieved to 2 mm and any visible plant materials were removed by hand.

4.2.2 Plant materials

All plant materials were collected from experimental plots used in the field component of this research. Corn and soybean materials were collected at harvest in mid-October and end-September, respectively. Red clover and timothy materials were collected at first cut in mid-June. Root sampling was done by digging with a shovel (20 cm diameter, 30 cm deep) around two random plant shoots in each plot. Collected samples were kept in the fridge for a maximum of 7 days before being washed free of soil with water. Root material was carefully washed to eliminate soil. All plant materials were dried in a forced draft oven at 70°C for a minimum of 48 h, or until constant weight, and were ground to pass through a 1 mm sieve.

4.2.3 Plant material characterization

Total carbon and nitrogen in plant materials were determined with an elemental Carlo Erba analyzer NCS 2500 (Thermo Quest Italia S.P.A., Rodano, Italy). The % fiber, % lignin and % hemicellulose were determined by the Van Soest procedure (Van Soest, 1963; Van Soest and Wine, 1967). Neutral and acid detergent fiber (NDF and ADF, respectively) were determined using the filter-bag technique and the ANKOM[200] fiber analyzer. Fiber content of plant materials was defined as being the neutral detergent fiber portion. Lignin content (acid detergent lignin) was determined using the beakers method as recommended by ANKOM Technology (ANKOM Technology, Fairport, New York, USA). The percentage hemicellulose was determined as being the difference between % NDF and % ADF. Percentage fiber, lignin and hemicellulose were expressed on a dry-mater basis. Initial chemical characteristics of incubated plant materials are presented in Table 4.1.

4.2.4 Incubation procedure and treatments

Nine treatments were applied: CL, corn (leaves+stems); CR, corn roots; SL, soybean (leaves+stems); SR, soybean roots; RCL, red clover (leaves+stems); RCR, red clover roots; TL timothy (leaves+stems); TR, timothy roots; and Soil, no plant material addition.

One week before material addition, the soil was moistened to 70% of field capacity and pre-incubated at 22°C. After soil pre-incubation, amendments were thoroughly mixed with soil at a rate equivalent to 0.1 g of carbon per 100 g soil dry weight. To avoid nitrogen limitation, ammonium sulphate was added to CR, CL, SR, SL and TR samples to bring the C/N ratio of

soil amendment to 25. Soil with amendments was incubated for a period of 3 days, 1, 2, 7, 13, 16 and 28 weeks at 22°C in 1 L mason jars covered with plastic bags to allow gas movement but limit water evaporation. The jars were weighed frequently and moisture content adjusted, if required, to 70% of field capacity. The jars were placed in an incubator room and organised in a randomized complete block design with three blocks and one replicate/treatment/sampling date/block.

4.2.5 Soil sampling

Approximately 50g of soil was first taken from the jar with a spoon for aggregate determination, carefully placed in a paper bag and air-dried until constant weight. The soil remaining in the jar was thoroughly homogenised, frozen and freeze dried at -80°C for at least three days prior to fatty acid extraction.

4.2.6 Soil aggregation

Approximately 40 g of air-dried soil were placed over the top of a nest of sieves (20.5 cm diameter) with openings of 4, 2, 1, 0.5 and 0.25 mm with a collecting pan at the bottom and a cover on top of the 4 mm sieve. The nest of sieves was placed on a Soil Test Engineering Equipment shaker model CL-305 A, and the soil was dry sieved by shaking for 2 minutes at 120 oscillations per minute. The weight of the fraction remaining on each sieve was recorded and the results were expressed as g of soil retained on the sieve per 100 g of total soil placed on the 4 mm sieve (% of total soil) on an air-dry basis. Mean Weight Diameter (MWD) of the dried aggregates was calculated as described by Kemper and Rosenau (1986). Macroaggregates were defined as the sum of aggregates greater than 0.25 mm in size and microaggregates were defined as the sum of aggregates smaller or equal to 0.25 mm in size. Change in soil structure variables (MWD, % micro and macroaggregates, 4, 2, 1, 0.5, 0.25 and <0.25 mm size aggregates) was calculated by subtracting the mean (n = 3) of the values obtained on the day of plant material addition to soil (day 0) from the values obtained at each sampling date (3 days, 1, 2, 7, 13, 16 and 28 weeks).

4.2.7 Microbial community structure

The microbial community structure was determined using fatty acid methyl ester (FAME) analysis. A modification of the method proposed by Schutter and Dick (2000) was used. Approximately 3 g of soil and 15 mL of 0.2 M KOH in methanol were incubated at 37ºC for an hour in screw-cap glass centrifuge tubes. During this step, ester-linked fatty acids were released and methylated. The pH of the tube content was neutralized by adding 3 mL of 1.0 M acetic acid and the FAMEs were partitioned into an organic phase by adding 15 mL of hexane:chloroform (4:1, v/v). The hexane layer was transferred to a clean glass tube and evaporated under a stream of N_2. In the final step, FAMEs were dissolved in 1 mL of iso-octane containing 24.5 ng/µl of nonadecanoate (C19:0) used as an internal standard.

FAMEs were analysed in split mode (100:1) using a Hewlett Packard 6890 Gas Chromatograph equipped with a 25 m Ultra2 column (cross-linked 5% diphenyl-95% di-methylpolysiloxane; internal diameter, 0.22 mm; film thickness, 0.33 µm; Agilent J&W 19091B-102) and flame ionization detector. The temperature was programmed to increase from 170ºC to 310ºC at a rate of 20ºC per min., and held at 310ºC for 1 min. Detector and inlet temperatures were 300ºC and 250ºC, respectively. Hydrogen was the carrier gas, nitrogen was the make-up gas and the flame was supported by air at a flow rate of 30, 30 and 400 mL min^{-1} respectively. We used the same setting as in the Microbial Identification System (MIS) protocol (MIDI, Inc., Newark, Delaware, USA, www.midi-inc.com) (Sasser, 1990; Schutter and Dick, 2000; Buyer, 2002). The retention times of the peaks were converted to equivalent chain length (ECL) values (Sasser, 1990). Peaks were identified based on their retention time (ECLs) as compared with commercial FAME standards (Supelco 37 Component FAME Mix cat.#47885-U; Supelco Bacterial Acid Methyl Esters cat.#47080-U; Matreya PUFA-2 cat.#1081; Matreya Bacterial Acid Methyl Esters CP Mix cat.#1114; Matreya cis-11-Hexadecenoic Acid cat.#1208 and Matreya 10-Methyloctadecanoate cat.#1763). This led to the identification of over 70 FAME. A subset of our samples was sent to a certified external laboratory (Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, QC) where samples were analysed using the Sherlock Microbial Identification System® (MIDI, Inc., Newark, Delaware, USA) and peak identifications were cross-validated.

Fatty acid nomenclature is of the form A:BωC where A designates the total number of carbon atoms, B the number of double bonds, and C the distance of the closest unsaturation

from the aliphatic end of the molecule. The suffixes c for *cis* and t for *trans* refer to geometric isomers. The prefixes "i" and "a" refer to iso and anteiso methyl-branching, respectively. Cyclopropane fatty acid is indicated by the prefix Cy, methyl and hydroxy groups are indicated by Me and OH, respectively. A total of 29 fatty acids were quantified to elaborate a FAME profile (Table 4.2). Total FAME content was expressed as nanograms FAME per gram dry soil and individual FAME abundance was expressed as a percentage of total FAME. Average proportions were determined using values obtained on the first day, 3 days, 1, 2, 7, 13, 16 and 28 weeks after the onset of incubation.

4.2.8 Statistical analyses

Statistics were performed using Statistix®8.0 for Windows. Analysis of variance was used to detect the effect of the treatments on soil structure variables and the LSD all-pair wise comparisons test was used for comparison of means with α=0.05. Redundancy analysis (RDA) was performed to visualize the relationship between microbial community structure (FAME profiles) and change in soil structure (MWD, macro and microaggregates and aggregates size fraction). RDA is a canonical form of principal component analysis (PCA) in which the axes are restricted to be linear combinations of explanatory variables; it helps to assess the relationship(s) between the explanatory variables and response variable values. In the resulting ordination diagram, the treatments are represented by points and explanatory and response variables are represented by arrows projecting from the origin. The response variable arrows point in the direction of maximum variation and their length is proportional to the rate of change and importance of the considered variable in explaining site differences. Longer explanatory variable arrows indicate a greater effect of that variable on the total response variable variation. The cosine of the angle formed by two arrows is an indication of the correlation between the two variables, a smaller angle indicating a greater correlation.

FAME data were used as explanatory variables and soil structure variables as response variables (called "species" in ordination models). Except for the first day of incubation, FAME and soil structure data obtained at all sampling dates were included in the RDA analysis. The focus of the ordination was on correlations between soil structure variables; the latter were centered and standardized. The Monte Carlo permutation test was performed to evaluate the significance of the relationship between FAME and soil structure variables. The test performed

199 unrestricted permutations under the reduced model. Monte Carlo test, PCA and RDA were performed using the program CANOCO for Windows (Version 4.0).

4.3 RESULTS AND DISCUSSION

4.3.1 Microbial community structure

Addition of plant materials had a large effect on the structure of the soil microbial community. Individual fatty acid analysis (Table 4.2) showed that 18 out of 29 selected FAMEs were significantly different in soil incubated without plant material as compared to all the other applied treatments. Over the entire period of incubation soil incubated without addition of plant material had a microbial community characterised by high proportions of bacterial FAME and low proportions of fungal FAME when compared to the other treatments. This is consistent with results obtained in other studies showing that addition of organic material produced changes in the structure of the microbial community with proportions of fungi increasing with time of decomposition (Nakamura et al., 2003; Aneja et al., 2004; Neely et al., 1991; Henriksen and Breland, 2002). The individual fatty acid analysis indicates that the microbial community composition differed with treatments (Table 4.2). Some treatments showed great similarity in composition when their total proportions of fungi, bacteria, Gram(-), Gram(+) and actinomycetes were considered over the entire period of incubation. Thus, (SL and CL), (RCR and RCL), (RCR and TR), and (CL and SR) had similar microbial community structure differing only in their proportions of bacteria, Gram(-) bacteria, actinomycetes and fungi, respectively.

4.3.2 Soil aggregation

Soil dry MWD and % macroaggregates differed among the treatments (Table 4.3), with some plant material additions resulting in greater MWD and % macroaggregates than with soil alone. Most differences that occurred were evident within 3 to 7 days.

Soybean leaf tissues induced the highest increase in soil aggregate and increased soil MWD and proportion of macroaggregates (% macroaggregates) as early as 3 days and 1 week after the onset of incubation (Table 4.3). The improvement in soil MWD was 1.6 to 7 times higher when soybean leaves were added as compared to soil without material addition and this was so at all dates during the incubation, except at 16 weeks. Soybean root addition increased MWD

by up to 4 times as compared to soil without material addition, but numerical differences were statistically significant only after 3 days of incubation. Corn, red clover and timothy material (roots and leaves) had no effect on soil MWD, when compared to soil incubated without material addition, from 3 days to 28 weeks after the onset of incubation. In an incubation experiment, Martens (2000) found that addition of residues from seven different crops resulted in an increase in soil MWD as early as 9 days following material addition for all residues. In Martens' study, the effect of the different plant materials on soil structure was more pronounced as incubation proceeded. In contrast to our findings, corn residues had a greater effect on soil MWD than soybean residues.

After 3 days of incubation, macroaggregation was only affected by addition of soybean roots (Table 4.3). After 1 week, there was an increase in the percentage of soil macroaggregates when soybean (roots and leaves) materials were added to soil. With soybean leaves, the increase in macroaggregation was detectable until 7 weeks after the onset of incubation. At this time, soybean and corn roots as well as red clover leaves also increased soil macroaggregation. Except for corn roots, it was also the case after 28 weeks of incubation, when the increase in macroaggregates was almost 5 times higher with addition of soybean leaves as compared to the soil incubated without organic material. As compared to soil with no material addition, corn and timothy materials had no effect on soil structure (MWD and macroaggregates) except on macroaggregation after 7 and 28 weeks of incubation with corn and timothy leaf additions, respectively.

When plant material addition effects are compared over the entire incubation period, SL seemed to be the treatment resulting in the best improvement in soil structure (MWD and macroaggregation) whereas CL and RCR seemed the least effective. The increase in soil macroaggregation was most responsive to treatment, and was a better indicator of treatment effects on soil structure than the increase in soil MWD, especially after 7 and 28 weeks of incubation. Seven weeks after the onset of incubation all treatments, except CL, RCR, TL and TR, had significantly improved the percentage of soil macroaggregates as compared to the soil incubated without material addition, while only SL significantly improved MWD.

4.3.3 Relationship between soil structure and microbial community composition

The relationship between microbial community structure (FAME profiles) and soil structure variables was highly significant (Figure 4.1; Monte Carlo test gives P = 0.005 for both the first and all canonical axes). Variable ranking by forward selection and the Monte Carlo permutation test showed that fungal marker fatty acid 18:1w9c had the greatest effect on soil structure variables and explained almost 14% (P = 0.005) of the total variance explained by all the explanatory variables. Fungi, in general, have a recognized effect on soil aggregation, particularly at the macroaggregate level. In the hierarchical conceptual model of Tisdall and Oades (1982), soil physical entanglement by fungal hyphae and fungal polysaccharides acting as binding agents play a major role in aggregation. The contribution of fungi to soil structure, particularly their effect on macroaggregation has been observed in many studies (e.g., Degens et al., 1996; Bossuyt et al., 2001). Fungal fatty acid 18:1ω9c correlated negatively with % microaggregates and aggregates smaller than 0.25 mm, while correlating positively with % macroaggregates. This agrees with the conceptual hierarchical model of aggregate formation proposed by Tisdall and Oades (1982) where microaggregates are brought together into macroaggregates by the enmeshing effect of fungal hyphae.

Gram(-) markers fatty acids Cy17:0 and Cy19:0 were also important and explained together 18% of the total variance (P = 0.005). Gram(-) marker fatty acids 18:1ω7c and 16:1ω7c were also significantly related to soil structure (P = 0.01 and 0.045, respectively). Both cyclopropyl Gram(-) fatty acids correlated negatively with % microaggregates and with aggregates smaller or equal to 0.25 mm while only Cy17:0 positively correlates with % macroaggregates. It has been suggested that macroaggregates contain more recent C than microaggregates (Puget et al., 1995; Angers and Giroux, 1996). If this true, we may then expect the microorganisms preferentially using more recent C, to be positively correlated with macroaggregates. Several studies have suggested that different groups of bacteria might have different preferences for carbon sources. Kramer and Gleixner (2006) used isotopic C and showed that Gram(-) bacteria preferentially used recent plant material as a carbon source, while Gram(+) bacteria used older soil organic matter. Similarly, in a study where labelled substrates were added to soil, Waldrop and Firestone (2004) found more ^{13}C incorporated into Gram(-) bacteria biomarkers than into Gram(+) biomarkers. The proportion of monounsaturated fatty acids, mainly from Gram(-) bacteria, was also found to increase while

the proportion of branched fatty acids, mainly from Gram(+) bacteria, decreased following substrate addition to soil (Phillips et al., 2002; Bossio and Scow, 1995). It seems that Gram(-) would be particularly responsive to fresh, newly added substrates. We may then hypothesize that Gram(-) bacteria could initiate the formation of macroaggregates by decomposing fresh, newly added organic material. If Gram(-) bacteria respond more rapidly to addition of new carbon sources than do Gram(+), when organic carbon is added to soil, Gram(-) bacteria growth should be stimulated and more extracellular polysaccharides should be produced by the microbes. The relationship between bacteria growth stage and extracellular polysaccharide production is not clear. Although polysaccharides appear to be produced during both the exponential and the stationary growth phase of bacteria, some studies have shown polysaccharide production to start with microbial growth but to be maximal in the stationary growth phase (Uhlinger and White, 1983; Barclay and Lewin, 1985; De and Basu, 1996). Others have found the greatest rate of extracellular polysaccharides during the log phase as compared to the stationary growth phase (Mian et al., 1978; Bramhachari and Dubey, 2006). When polysaccharide production rate is at its maximum, the number of bacterial cells is also likely to be maximal. Extracellular polysaccharides, may establish chemical interactions with soil particles and initiate the formation of macroaggregates.

Gram(-) markers Cy17:0, Cy19:0, 18:1ω7c and 16:1ω7c have also been related to the physiological status of Gram(-) bacteria. Some studies have shown that monoenoic fatty acids, 16:1ω7c and 18:1ω7c, are increasingly converted to the cyclopropyl fatty acids, Cy17:0 and Cy19:0, when Gram(-) bacteria switch from an exponential to stationary growth phase (Tunlid et al., 1985; Guckert et al., 1986). The fact that no bacterial marker other than cyclopropyl fatty acids and their precursors are significant in explaining soil structure suggested that cyclo/precursors ratio, and hence the physiological or nutritional status of bacteria, might be important in explaining variation in soil structure variables.

This analysis showed that microbial community composition could explain up to 24% of the variation in soil structure. Although microbial community size is not always found to be correlated with soil structure, given a particular microbial community composition, variation in soil structure among applied treatments could also be explained by differences in microbial community density. Furthermore, differences in the chemistry of decomposing materials

(Table 4.1) and their degradation products might affect their interactions with soil particles and thus be responsible for part of the variation in soil structure among treatments.

4.4 CONCLUSION

Our results show that introducing plant materials differing in quality into the soil affects the soil microbial community structure and increases soil dry MWD and % macroaggregates to varying degrees.

The size of the microbial community, expressed as total FAMEs, was not correlated with soil structure, but we found significant relationships between microbial community FAME composition and the size distribution of soil aggregates. The soil microbial community structure was found to explain only 24% of the variation in soil structure but this might have been underestimated since there might be a delay between changes in the composition of the microbial community and the actual effect of microorganisms on soil structure.

Our results are in agreement with the recognized role of fungi in macroaggregate formation and suggest that Gram(-) bacteria might be involved in the formation of macroaggregates . The lack of available studies to corroborate our findings points out the need for new research on microbial community structure and diversity among aggregates size classes. Indeed, if soil microorganisms can be manipulated to improve soil tilth, the identification of species or groups of microorganisms with the best aggregating abilities is an area of research that should be further explored. Identifying microorganisms involved in microaggregate formation and stabilization is of great interest if we want to increase the amount of carbon stored in the soil, since microaggregates are thought to be more stable and to contain older and more stabilized OM than macroaggregates.

Table 4.1 : Initial chemical characteristics of incubated plant material

Material	C/N	% hemicellulose	% fiber	% lignin	lignin/N
Corn leaves+stems	72a[†]	33a	81a	6f	11c
Corn roots	43c	31b	77a	11de	10cd
Red clover leaves+stems	17fg	10f	47de	15c	7de
Red clover roots	14g	13e	43e	13cd	5ef
Soybean leaves+stems	48b	16d	63c	10e	11c
Soybean roots	34d	17d	79a	20b	16b
Timothy leaves	20f	21c	49d	6f	3f
Timothy roots	26e	20c	68b	29a	24a

[†]Numbers within a column followed by the same letter(s) are not significantly different at the 0.05 probability level.

Table 4.2: Fatty acid methyl esters (FAME) extracted from soil treated or not with different plant material and their mean relative abundance of all sampling dates during the incubation period (n=24).

Fatty acid	Marker	CL[†]	CR	RCL	RCR	SL	SR	TL	TR	Soil
		Average proportion (% total FAME)								
Bacteria[a]		35.1g[b]	38.8de	42.3bc	41.1bc	37.3ef	36.5fg	40.6cd	42.7b	46.2a
15:0	Bacteria[e]	1.0cd	1.1bcd	1.1bcd	1.1bc	1.1bcd	1.1bcd	1.0d	1.2b	1.4a
17:0	Bacteria[e]	0.6cd	0.7abc	0.8ab	0.8a	0.8ab	0.8ab	0.7abc	0.7bc	0.8a
Gram (-)		13.2ef	14.4cd	16.8a	15.2bc	13.8de	12.5f	15.8ab	15.9ab	15.2bc
16:1ω7c	Gram(-)[c]	2.9d	3.2bcd	3.3bc	3.7a	3.1cd	2.5e	3.1cd	3.5ab	2.9d
16:1ω11c	Gram(-)[g]	0.6cd	0.7abc	0.7abc	0.7ab	0.6bc	0.5d	0.6bc	0.8a	0.7abc
18:1ω5c	Gram(-)[c]	1.8c	1.9bc	4.3a	1.9bc	1.9bc	1.4d	4.1a	2.1b	2.1bc
18:1ω7c	Gram(-)[c]	4.4c	4.8abc	4.6bc	5.1ab	4.6bc	4.2c	4.4c	5.3a	4.6bc
Cy17:0	Gram(-)[c]	1.2e	1.3cd	1.3bc	1.3bc	1.2de	1.2de	1.2de	1.4b	1.5a
Cy19:0	Gram(-)[c]	0.8ef	1.0cd	0.9de	1.9a	1.0c	2.0a	0.8f	1.0cd	1.3b
Gram (+)		20.6g	22.4def	23.5bcd	23.6bc	21.2fg	22.0ef	22.8cde	24.6b	29.1a
i-14:0	Gram(+)[g]	0.6e	0.7bcd	0.7b	0.7bc	0.6de	0.7cde	0.7bcd	0.7bc	0.9a
i-15:0	Gram(+)[c]	4.8de	5.2c	5.1cd	5.1cd	4.8e	5.2c	4.9cde	5.7b	7.1a
a-15:0	Gram(+)[c]	3.0f	3.3e	4.0ab	4.1a	3.4de	3.2ef	3.8bc	3.6cd	4.1a
i-16:0	Gram(+)[c]	2.7e	2.9de	3.3b	3.3b	2.8de	3.0cd	3.3b	3.2bc	3.7a
i-17:0	Gram(+)[c]	1.9d	2.1c	2.0cd	2.0cd	1.9d	2.1c	2.2bc	2.3b	2.9a
a-17:0	Gram(+)[c]	1.9d	2.1cd	2.4b	2.4b	2.1c	2.1c	2.4b	2.4b	2.7a
10Me16:0	Gram(+)[c], Actinomycetes[d]	5.6d	6.2c	5.9cd	5.8d	5.6d	5.7d	5.6d	6.8b	7.8a
Actinomycetes		7.7e	8.5c	8.4c	8.2cd	7.8de	7.9de	7.9de	9.2b	10.3a
10Me17:0	Actinomycetes[e]	0.4cde	0.4de	0.6a	0.6ab	0.5bc	0.5bc	0.6ab	0.5bcd	0.4e
10Me18:0	Actinomycetes[d]	2.3e	2.6c	2.5c	2.5cd	2.4de	2.6c	2.3e	2.8b	3.4a
Fungi		30.9a	26.6bc	23.3de	23.3de	28.5ab	25.8cd	26.9bc	21.5e	13.3f
16:1ω5c	Mycorrhizal Fungi[f]	2.1c	2.4c	2.4c	2.6b	2.2c	2.1c	2.2c	2.9a	2.8ab
18:1ω9c	Fungi[g,c]	8.9b	8.5b	11.3a	8.7b	8.8b	7.9b	10.8a	7.9b	5.8c
18:2ω6,9c	Fungi[f,c]	19.2a	15.1bc	9.1f	11.6de	17.0ab	15.1bc	13.5cd	10.2ef	3.7g
20:1ω9c	Fungi[f]	1.5de	1.5d	1.6d	1.6d	1.5de	1.9b	1.4e	1.7c	2.1a
14:0	Not specific	1.5cd	1.6bcd	1.6bc	1.7b	1.5d	1.6bc	1.6bc	1.7b	2.3a
16:0	Not specific	15.0c	14.9c	15.0c	15.1c	14.9c	15.8ab	14.2d	15.5bc	16.1a
18:0	Not psecific	0.6abc	0.7abc	0.5c	0.5bc	0.8ab	0.7ab	0.5c	0.8a	0.8a
20:0	Not specific	4.4c	4.5bc	4.0d	4.0d	4.3c	4.6b	3.9d	4.5bc	4.9a
17:1ω8c		0.8c	1.0b	1.1ab	1.1a	1.0ab	0.8c	1.1ab	1.0ab	0.8c
16:1-2OH		2.9def	3.1cd	2.9de	2.9def	2.8ef	3.2bc	2.7f	3.4b	4.4a
17:0-2OH		1.7e	1.8bc	1.8bc	1.8bc	1.7de	1.8cde	1.8cd	1.9b	2.2a
18:0-2OH		0.7b	0.6bc	0.5bcd	0.5cd	0.5bcd	0.7bc	0.4d	0.6bcd	1.0a

[†]CL, corn (leaves+stems); CR, corn roots; SL, soybean (leaves+stems); SR, soybean roots; RCL, red clover (leaves+stems); RCR, red clover roots; TL timothy (leaves+stems); TR, timothy roots; and Soil, no material addition. [a]Average bacteria proportions include general bacterial markers, Gram(+) and Gram(-) markers. [b]Numbers within a row followed by the same letter(s) are not significantly different at the 0.05 probability level. [c]Zak et al., 1996; [d]White et al., 1997; [e]Ritchie et al., 2000; [f]Madan et al., 2002; [g]Zelles 1997

Table 4.3: Effects of plant materials on the change in mean-weight diameter (MWD) of dry soil stable aggregates and percentage of soil included in dry macroaggregates (>0.25 mm).

	Time since addition of plant material to soil						
	3 days	1 week	2 weeks	7 weeks	13 weeks	16 weeks	28 weeks
Plant materials	MWD (mm)						
CL[†]	0.01d[‡]	0.04bc	0.05bcd	0.04b	0.07cd	0.06bc	0.03bc
CR	0.09bcd	0.12abc	0.08bc	0.09ab	0.09bcd	0.09abc	0.05bc
RCL	0.13abc	0.10abc	0.10b	0.15ab	0.14abc	0.14ab	0.12ab
RCR	0.02d	0.03c	0.02d	0.06ab	0.04d	0.03c	0.00c
SL	0.16ab	0.20a	0.22a	0.19a	0.20a	0.19a	0.21a
SR	0.19a	0.14ab	0.08bc	0.15ab	0.16ab	0.15ab	0.08bc
TL	0.08bcd	0.08bc	0.08bcd	0.07ab	0.04d	0.08abc	0.08bc
TR	0.05cd	0.08bc	0.08bc	0.06ab	0.06d	0.07abc	0.12ab
Soil	0.05cd	0.05bc	0.03cd	0.04b	0.10bcd	0.09abc	0.05bc
	Macroaggregates (%)						
CL	1.9bc	0.9d	1.1bc	0.8d	1.7c	2.1bc	0.8d
CR	4.2ab	3.5abc	2.5bc	3.6abc	3.9abc	3.7ab	2.5bcd
RCL	4.5ab	3.5abc	3.2ab	4.6ab	4.2ab	4.4ab	3.6bc
RCR	1.0d	0.8d	0.4c	2.3cd	2.0bc	1.4c	1.3d
SL	4.8ab	5.4a	5.2a	5.6a	5.6a	6.1a	6.7a
SR	5.3a	4.1ab	3.3ab	4.2abc	4.6a	4.4ab	3.7b
TL	3.5abc	2.2bcd	3.0ab	2.2cd	2.1bc	3.1bc	2.4bcd
TR	1.9cd	2.1bcd	2.1bc	2.7bcd	1.7c	3.1bc	3.8b
Soil	2.9bcd	1.8cd	1.8bc	1.4d	3.9abc	2.9bc	1.4cd

[†]CL, corn (leaves+stems); CR, corn roots; SL, soybean (leaves+stems); SR, soybean roots; RCL, red clover (leaves+stems); RCR, red clover roots; TL timothy (leaves+stems); TR, timothy roots; and Soil, no material addition. [‡]Numbers within a column followed by the same letter(s) are not significantly different at the 0.05 probability level.
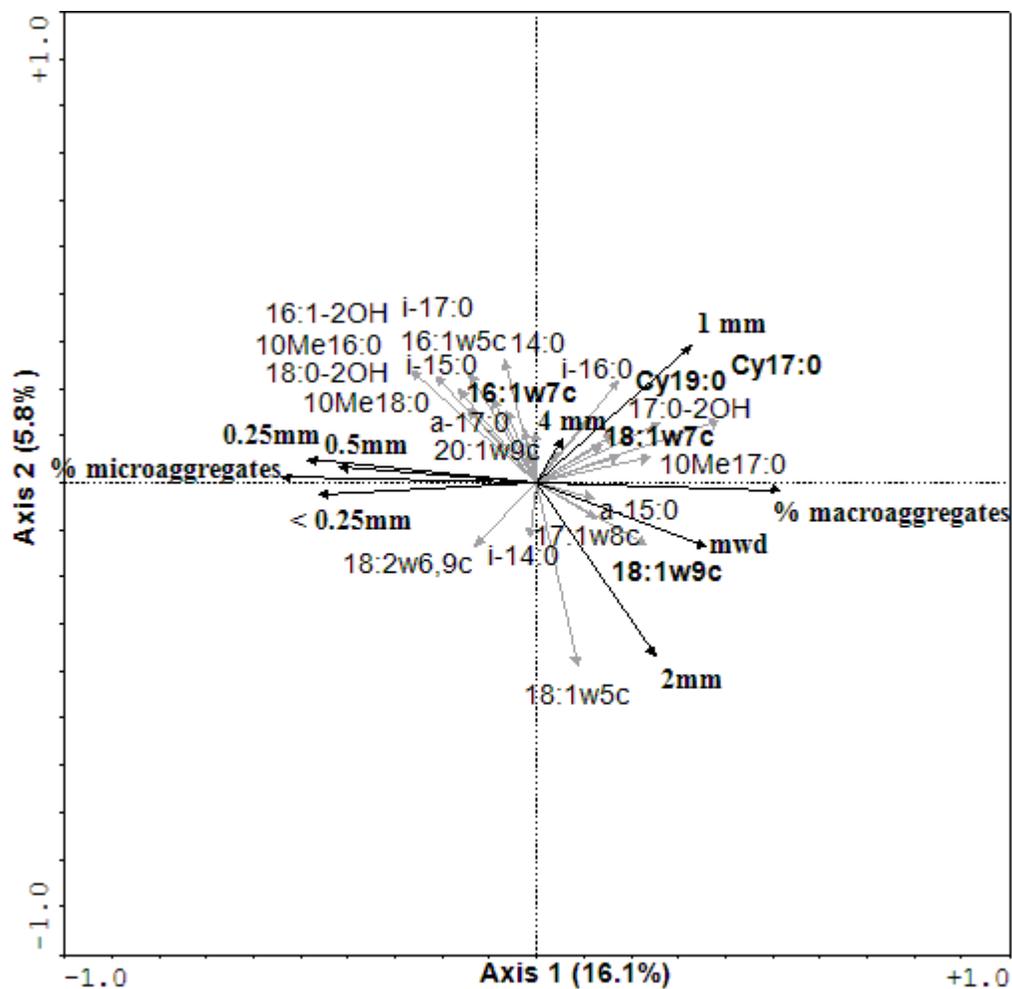
Figure 4.1: Redundancy analysis (RDA) exploring the relationship between FAME profiles and change in soil structure variables from 3 days until 28 weeks after amendments addition to soil. The 29 FAMEs given in Table 4.2 were used as explanatory variables and change in soil structure variables as species variables. Percentages represent the amount of variability explained by the axis.

REFERENCES

Allison, V.J., Miller, R.M., Jastrow, J.D., Matamala, R. and Zak, D.R. 2005. Changes in Soil Microbial Community Structure in a Tallgrass Prairie Chronosequence. Soil Science Society of America Journal 69:1412–1421

Aneja, M.K., Sharma, S., Munch, J.C. and Schloter, M. 2004. RNA fingerprinting—a new method to screen for differences in plant litter degrading microbial communities. Journal Of Microbiological Methods 59:223–231.

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

Angers, D.A., Pesant, A. and Vigneux, J. 1992. Early cropping-induced changes in soil aggregation, organic matter and microbial biomass. Soil Science Society of America Journal 56:115-119.

Barclay, W.R. and Lewin, R.A. 1985. Microalgal polysaccharide production for the conditioning of agricultural soils. Plant and Soil 88: 159-169

Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C. 1999. Seasonal changes in microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry 31: 1021-1030

Bossio, D.A. and Scow, K.M. 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied Environmental Microbiology 61:4043–4050

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R. and Paustian, K. 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16: 195-208.

Bramhachari, P.V. and Dubey, S.K. 2006. Isolation and characterization of exopolysaccharide produced by Vibrio harveyi strain VB23. Letters in Applied Microbiology 43: 571–577

Buyer, J. 2002. Identification of bacteria from single colonies by fatty acid analysis. Journal of Microbiological Methods 48:259-265.

Calderon, F.J., Jackson, L.E., Schow, K.M. and Rolston D.E. 2001. Short-term dynamics of nitrogen, microbial activity and phospholipids fatty acids after tillage. Soil Science Society of America Journal 65:118-126.

Carter, M.R., Angers, D.A. and Kunelius, H.T. 1994. Soil structural form and stability and organic matter under cool season perennial grasses. Soil Science Society of America journal 58:1194-1199.

Chantigny, M.H., Angers, D.A., Prevost, D., Vezina, L-P. and Chalifour F-P. 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Science Society of America Journal 61:262-267.

Cheshire, M.V. 1979. Nature and origin of carbohydrates in soils. Academic Press, London.

De, P.S. and Basu, P.S. 1996. Production of extracellular polysaccharides by a *Rhizobium* species from the root nodules of *Tephrosia purpurea* Pers. Acta Biotechnologica 16:155-162.

Degens, B.P., Sparling, G.P. and Abbott, L.K. 1994. The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long term clover-based and grass pastures. European Journal of Soil Science 45: 459-468.

Degens, B.P., Sparling, G.P. and Abbott, L.K. 1996. Increasing the length of hyphae in a sandy soil increases the amount of water-stable aggregates. Applied Soil Ecology 3:149-159.

Drury, C.F., Stone, J.A. and Findlay, W.I. 1991. Microbial biomass and soil structure associated with corn, grasses, and legumes. Soil Science Society of America Journal 55: 805-811.

Griffiths, E. and Burns, R.G. 1972. Interaction between phenolic substances and microbial polysaccharides in soil aggregation. Plant and Soil 36: 599-612.

Guckert, J.B., Hood, M.A. and White, D.C. 1986. Phospholipid, ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increase in the *trans/cis* ratio and proportions of the cyclopropyl fatty acids. Applied Environmental Microbiology 52:794-801.

Hebbar, K.P., Gueniot, B., Heyraud, A., Colin-Morel, P., Heulin, T., Balandreau, J. and Rinaudo, M.1992. Characterization of exopolysaccharides produced by rhizobacteria. Applied Microbiology and Biotechnology 38(2) : 248-253

Henriksen, T.M., and Breland, T.A. 2002. Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil. Biology and Fertility Of Soils 35:41–48.

Ibekwe, A.M. and Kennedy, A.C. 1999. Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant and Soil 206:151-161

Kramer, C. and Gleixner, G. 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. Soil Biology and Biochemistry 38: 3267-3278.

Lynch, J.M. and Bragg, E. 1985. Microorganisms and soil aggregate stability. Advances in Soil Science 2: 134-170

Madan, R., Pankhurst, C., Hawke, B. and Smith, S. 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. Soil Biology and Biochemistry 34: 125-128.

Martens, D.A., 2000. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. Soil Biology and Biochemistry 32: 361-369.

Martin, J.P. 1942. The effect of compost and compost materials upon the aggregation of the silt and clay particles of Collington sandy loam. Soil Science Society of America Proceedings 7: 218-222.

Martin, J.P. and Waksman, S.A. 1941. Influence of microorganisms on soil aggregation and erosion. II. Soil Science 52: 381-394.

Mian, F.A., Jarman, T.R. and Righelato, R.C. 1978. Biosynthesis of exopolysaccharide by *Pseudomonas aeruginosa.* Journal of Bacteriology 134:418-422

Nakamura, A., Tun, C.C., Asakawa, S. and Kimura, M. 2003. Microbial community responsible for the decomposition of rice straw in a paddy field: Estimation by phospholipids fatty acid analysis. Biology and Fertility of Soils 38:288–295.

Neely, C.L., Beare, M.H., Hargrove, W.L. and Coleman, D.C. 1991. Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. Soil Biology and Biochemistry 23:947–954.

Oades, J. M. and Waters, A.G. 1991. Aggregate hierarchy in soils. Australian Journal of Soil Research 29: 815-828.

Oades, J.M., 1984. Soil organic matter and structural stability mechanisms and implications for management. Plant and Soil 76: 319-337

Perfect, E., Kay, B.D., Van Loon, W.K.P., Sheard, R.W. and Pojasok, T. 1990. Factors influencing soil structure stability within a growing season. Soil Science Society of America Journal, 54: 173-179.

Phillips, R.L., Zak, D.R., Holmes, W.E. and White D.C. 2002. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131:236–244

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

Ritchie, N. J., Schutter, M.E., Dick, R.P. and Myrold, D.D. 2000. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied and Environmental Microbiology 66 (4): 1668-1675.

Robertson, E.B., Sarig, S. and Firestone, M.K. 1991. Cover crop management of polysaccharides-mediated aggregation in an orchard soil. Soil Science Society of America Journal 55:734-739.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Tech. Note #101. Microbial ID, Newark, USA.

Schutter, M.E., and Dick, R.P. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64:1659-1668.

Sutherland, I.W. 1985. Biosynthesis and composition of Gram negative bacterial extracellular and wall polysaccharides. Annual Review of Microbiology 39: 243-270

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

Tisdall, J.M., 1994. Possible role of soil microorganisms in aggregation in soils. Plant and Soil 159: 115-121.

Tunlid, A., Baird, B.H., Trexler, M.B., Olsson, S., Findlay, R.H., Odham, G. and White, D.C. 1985. Determination of phospholipids ester-linked fatty acids and poly beta hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* (L). Canadian Journal of Microbiology 31:1113-1119.

Uhlinger, D. J. and White, D.C.1983. Relationship Between Physiological Status and formation of extracellular polysaccharide glycocalyx in *Pseudomonas Atlantica*. Applied and Environmental Microbiology 45 (1): 64-70.

Van Soest, P.J. and Wine, R.H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. Journal of the Association of Official Analytical Chemists 50: 50-55.

Van Soest, P.J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Journal of the Association of Official Analytical Chemists 46: 828-835.

Waldrop, M.P. and Firestone, M.K. 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138: 275–284

White, D.C., Stair, J.O. and Ringelberg, D.B. 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. Journal of Industrial Microbiology 17: 185-196.

Zak, D.R. Ringelberg, D.B., Pregitzer, K.S., Randlett, D.L., White, D.C. and Curtis, P.S. 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecological Applications 6: 257-262.

Zelles, L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35: 275-294.

Connecting paragraph

The multi-cropping systems field experiment presented in Chapter 3 suggested that microbial community structure affects soil structure. The composition of the microbial community was shown to be affected mainly by root biomass and lignin content. Chapter 4 presented the results of an incubation experiment where macroaggregation appeared to be affected by soil fungal proportion and suggested Gram(-) bacteria to also positively influence macroaggregation. Chapter 5 presents results obtained from the same incubation experiment and explores the relationship between microbial community structure and initial quality of incubated plant material.

Chapter 5

Microbial community structure dynamics during plant material decomposition

5.1 INTRODUCTION

The process of organic residue decomposition has been extensively studied. The biochemistry of organic material influences its decomposition rate along with environmental conditions such as soil temperature and moisture (Berg and McClaugherty, 2003). Decomposition appears to be a sequential process during which the quality of the decomposing material changes with time as more easily broken-down compounds are degraded first and more recalcitrant compounds are left behind. Several models have been proposed to describe the quality factors controlling the rate of decomposition.

It has been shown that soluble and simple compounds such as sugars are used first as an energy source by microorganisms and control the early stage of decomposition while more recalcitrant compounds such as lignin control the later stage of decomposition (Berg and McClaugherty, 2003). Changes in the biochemical nature of the decomposing material should drive a microbial succession with increasing ability to decompose complex compounds. Increase in the proportion of fungi relative to bacteria has been detected as decomposition proceeds and has been attributed to an increase in the complexity of the substrate (e.g., McMahon et al., 2005). However, some studies have also shown no changes and even a decline in the proportion of fungi during decomposition (Broder and Wagner, 1988; Lundquist et al., 1999). These uncertain relationships raise questions regarding the link between organic material quality and the composition of the microbial community. Little is known about how bacteria respond to the quality of organic inputs but it has been suggested that they are less affected by the quality of the substrate than fungi (Wardle, 1995). To our knowledge, no studies have yet clearly identified the organic material quality factors determining the composition of the microbial community.

Fatty acid profiling can be used to study microbial community structural changes. Fatty acid analysis has been successfully used to detect changes in microbial communities in

response to environmental conditions such as C and O_2 limitations, high temperature, acidic conditions (Knivett and Cullen 1965; Allison et al., 2005) and substrate availability (Thomas and Batt, 1969; Zelles et al., 1992; Bossio and Scow, 1998). Microbial fatty acids have also been shown to respond to changes in nitrogen fertilization (Bardgett et al, 1999), plant species (Ibekwe and Kennedy, 1999) and tillage (Drijber et al., 2000; Calderon et al 2001) as well as various management practices (Klug and Tiedje, 1993; Pankhurst, 1997). Shifts in microbial community fatty acids during organic material decomposition have also been observed in some studies (e.g., Sharma et al., 1998, Schutter and Dick, 2001).

In this experiment we incubated eight different crop materials under the same environmental conditions. Our objective was to determine how the initial chemical characteristics of decomposing plant material influence soil microbial populations. We hypothesize that the soil microbial community structure will reflect the initial chemical characteristics of the incubated materials but as decomposition proceeds, the differences in the microbial communities growing on the various crop materials will become less important. We expect more easily decomposable compounds to be responsible for early changes in microbial community structure and more recalcitrant compounds to affect the microbial community in the late stage of decomposition.

5.2 MATERIALS AND METHODS

5.2.1 Soil

The soil was collected from the surface layer (0-20 cm) of a field located at Ste-Anne-de-Bellevue, Quebec (45°25' N, 73°57' W). The soil was a Chateauguay clay loam with 43.4% sand, 22.6% clay and 34% silt, a pH of 7.8, 40 g C kg^{-1} and 3.0 g N kg^{-1} for a C/N ratio of 13. The soil was sieved to 2 mm and any visible plant materials were removed by hand. The soil was stored in dry conditions at room temperature for one year prior to the experiment.

5.2.2 Plant materials

All plant materials were collected from experimental plots used in the field component of this research. Corn and soybean materials were collected at harvest in mid-October and end-September, respectively. Red clover and timothy materials were collected at first cut in mid-

June. Root sampling was done by digging with a shovel (20 cm diameter, 30 cm deep) around two random plant shoots in each plot. Collected samples were kept in the fridge for a maximum of 7 days before being washed free of soil with water. Root material was carefully washed to eliminate soil. All materials were dried in a forced draft oven at 70°C for a minimum of 48 h, or until constant weight, and were ground to pass through a 1 mm sieve.

5.2.3 Plant materials characterization

Total carbon and nitrogen in plant materials were determined with an elemental Carlo Erba analyzer NCS 2500 (Thermo Quest Italia S.P.A., Rodano, Italy). The % fiber, % lignin and % hemicellulose were determined by the Van Soest procedure (Van Soest, 1963; Van Soest and Wine, 1967). Acid and neutral detergent fiber (ADF and NDF, respectively) were determined using the filter-bag technique and the ANKOM[200] fiber analyzer. Fiber content of plant materials was defined as being the neutral detergent fiber portion. The % fiber includes cellulose, hemicellulose and lignin, and represents the non-soluble fraction remaining after treatment with neutral detergent has removed soluble cell components such as simple sugars and amino acids from the treated material. Lignin content (acid detergent lignin) was determined using the beakers method as recommended by ANKOM Technology (ANKOM Technology, Fairport, New York, USA). The percentage hemicellulose was determined as the difference between % NDF and % ADF. Percentage fiber, lignin and hemicellulose were expressed on a dry-matter basis.

5.2.4 Incubation procedure and treatments

Nine treatments were applied: CL (corn leaves+stems), CR (corn roots), SL (soybean leaves+stems), SR (soybean roots), RCL (red clover leaves+stems), RCR (red clover roots), TL (timothy leaves+stems), TR (timothy roots) and Soil (no plant material addition).

One week before material addition, the soil was moistened to 70% of field capacity and pre-incubated at 22°C. After soil pre-incubation, amendments were thoroughly mixed with soil at a rate equivalent to 0.1 g of carbon per 100 g dry soil. To avoid nitrogen limitation, ammonium sulphate was added to CR, CL, SR, SL and TR samples to bring the C/N ratio of the sample to 25. Soils with amendments were incubated for a period of 3 days, 1, 2, 7, 13, 16 or 28 weeks at 22°C in 1 L mason jars covered with plastic bags to limit water evaporation but

allow gas movement. The jars were weighed frequently and moisture content readjusted to 70% field capacity. The samples were organised as a randomized complete block design with 3 blocks and one replicate/treatment/sampling date/block.

5.2.5 Soil sampling

At the end of each incubation period, the soil was thoroughly homogenised, frozen at -80°C and freeze dried for at least three days prior to fatty acid extraction.

5.2.6 Microbial community structure

The microbial community structure was determined using fatty acid methyl ester analysis (FAME) using a modification of the method proposed by Schutter and Dick (2000). Approximately 3 g of soil and 15 mL of 0.2 M KOH in methanol were incubated at 37ºC for an hour in screw-cap glass centrifuge tubes. During this step, ester-linked fatty acids were released and methylated. The pH of the tube content was neutralized by adding 3 mL of 1 M acetic acid and the FAMEs were partitioned into an organic phase by adding 15 mL of hexane:chloroform (4:1, v/v). The hexane layer was transferred to a clean glass tube and evaporated under a stream of N_2. In the final step, FAMEs were dissolved in 1 mL of iso-octane containing 24.5 ng/μL of nonadecanoate (C19:0) used as an internal standard.

FAMEs were analysed in split mode (100:1) using a Hewlett Packard 6890 Gas Chromatograph equipped with a 25 m Ultra2 column (cross-linked 5% diphenyl-95% di-methylpolysiloxane; internal diameter, 0.22 mm; film thickness, 0.33 μm; Agilent J & W 19091B-102) and flame ionization detector. The temperature was programmed to increase from 170ºC to 310ºC at a rate of 20ºC per min., and held at 310ºC for 1 min. Detector and inlet temperatures were 300ºC and 250ºC, respectively. Hydrogen was the carrier gas, nitrogen was the make up gas and the flame was supported by air at a flow rate of 30, 30 and 400 mL min^{-1}, respectively. We used the same setting as the Microbial Identification System (MIS) protocol (MIDI, Inc., Newark, Delaware, USA, www.midi-inc.com) (Sasser, 1990; Schutter and Dick, 2001; Buyer, 2002). The retention times of the peaks were converted to equivalent chain length (ECL) values (Sasser, 1990). Peaks were identified based on their retention time (ECLs) as compared with commercial FAME standards (Supelco 37 Component FAME Mix cat.#47885-U; Supelco Bacterial Acid Methyl Esters cat.#47080-U; Matreya PUFA-2 cat.#1081; Matreya

Bacterial Acid Methyl Esters CP Mix cat.#1114; Matreya cis-11-Hexadecenoic Acid cat.#1208 and Matreya 10-Methyloctadecanoate cat.#1763). This led to the identification of more than 70 FAME. A subset of our samples was sent to a certified external laboratory (Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, QC) where samples were analysed using the Sherlock Microbial Identification System® (MIDI, Inc., Newark, Delaware, USA) and peak identifications were cross-validated.

Fatty acid nomenclature is of the form A:BωC where A designates the total number of carbon atoms, B the number of double bonds, and C the distance of the closest unsaturation from the aliphatic end of the molecule. The suffixes c for *cis* and t for *trans* refer to geometric isomers. The prefixes "i" and "a" refer to iso and anteiso methyl-branching, respectively. Cyclopropane fatty acid is indicated by the prefix Cy, methyl and hydroxy groups are indicated by Me and OH, respectively. A total of 29 fatty acids were quantified to elaborate a FAME profile (Table 5.1). Total FAME content was expressed as nanograms FAME per gram dry soil, individual FAME abundance was expressed as a percentage of total FAME and abundance of each microbial group (Gram(+) and Gram(-) bacteria, actinomycetes and fungi) was expressed as the sum of the proportion of the individual FAME used as marker for that group of microorganisms. The FAMEs considered in this study are listed in Table 5.1.

5.2.7 Statistical analyses

Statistics were performed using Statistix®8.0 for Windows. The LSD all-pair wise comparison test was used for comparison of means with α=0.05. Correlations between variables were tested using Pearson's *r* coefficient. Principal component analysis (PCA) was used to determine if the applied treatments induced changes in microbial community structure (FAME profile) as decomposition proceeded. Redundancy analysis (RDA) was used to visualize the relationship between treatments, FAME and the initial quality of incubated materials. RDA is a canonical form of PCA in which the axes are restricted to be linear combinations of explanatory variables. This approach is used to assess the relationship(s) between the explanatory variables and response variable values. In the resulting ordination diagram, the treatments are represented by points and explanatory and response variables are represented by arrows projecting from the origin. The response variable arrows point in the direction of maximum variation and their length is proportional to the rate of change and

importance of the considered variable in explaining differences between samples. Longer explanatory arrows indicate a greater effect. The cosine of the angle formed by two arrows is an indicator of the correlation between the two variables, a smaller angle indicating a greater correlation.

FAME data were used as response variables (called "species" in ordination models) and material quality variables were used as explanatory variables. FAME data from soil incubated without material addition were not included in this analysis. Samples and FAMEs were centered and the focus of the ordination was on correlations between FAMEs. The Monte Carlo permutation test was performed to evaluate the significance of the relationships between FAMEs and material quality variables. The test performed 199 unrestricted permutations under the reduced model. Monte Carlo test, PCA and RDA were performed using the program CANOCO for Windows (Version 4.0).

5.3 RESULTS AND DISCUSSION

5.3.1 Changes in microbial community structure over time

Most of the changes in FAME profiles of amended treatments occurred within the first two weeks. After seven weeks, there were few changes in the structure of the microbial community (Figure 5.1). It seems likely that the microbial community took 1 to 2 weeks to adapt to their various substrates, after which there were changes in size but not in composition of the microbial community. For most of the treatments, FAME group analysis (% branched, monounsaturated, polyunsaturated, hydroxy, straight, fungal, bacterial, AMF, cyclo/precursors and saturated/unsaturated) showed that by 2 to 7 weeks, the microbial community composition was established and changed little thereafter (results not shown). Regardless of the treatment applied, late sampling dates were correlated with fungal marker 18:2ω6,9c (Figures 5.1a to 5.1i) suggesting that the proportion of fungi increased as decomposition proceeded. The increase in proportion of fungi with time is consistent with their role in the degradation of complex compounds (Schimel, 1995; Myers et al., 2001; Henriksen and Breland, 2002). Shifts in microbial community composition and increases in relative proportion of fungi as compared to bacteria have been observed in other studies as plant material decomposes (Nakamura et al., 2003; Aneja et al., 2004; Neely et al., 1991; Henriksen and Breland, 2002).

In most of the treatments, late sampling dates were also correlated with actinomycetes markers, in particular 10Me17:0 (Figure 5.1). Actinomycetes are a diverse group of Gram(+) bacteria able to produce depolymerization enzymes for recalcitrant compounds (Paul and Clark, 1996) which is consistent with their increased proportions as decomposition proceeds.

5.3.2 Differences in microbial community structure between treatments

Few differences in microbial community structure between treatments were detected at Day 0 of incubation (Figure 5.2a). Most of the samples are grouped together at the center of the diagram, indicating that they have similar microbial community structure although SR, Soil, RCL, TL, are different from the others. Most of the variation in FAME profiles measured at Day 3 may reflect the FAME composition of added materials (Figure 5.2b). For instance, RCL and TL correlate with 18:1ω5c reflecting the high levels of 18:1ω5c measured in red clover and timothy leaf materials (results not shown). After one week of incubation (Figure 5.2c), the samples began to diverge from each other, indicating that their microbial communities had begun to change. After two weeks (Figures 5.2d to 5.2h), some patterns began to emerge and these remained until the end of the incubation period. Fatty acid 18:2ω6,9c, which is commonly used as signature fatty acid for fungi (Zogg et al., 1997; Madan et al., 2002), was strongly correlated with PC 1. Except for fatty acid 18:1ω9c, which has also been used as a fungal biomarker (Zogg et al., 1997; Madan et al., 2002), most of the other fatty acids (mainly from bacteria) were on the opposite side of PC 1 suggesting microbial communities characterized by a greater proportion of fungi (SR, SL, CR and CL) at one end and a greater proportion of bacteria (Soil) at the opposite end of PC 1. The lower quality of soybean and corn materials (high C/N ratio and fiber content) might be responsible for the higher proportion of fungi in SL, SR, CR, and CL as compared to the other treatments (Table 5.2). Indeed, complete degradation of fiber is carried out mainly by fungi (Ericksson et al., 1990; Malherbe and Cloete, 2002; Berg and McClaugherty, 2003) and their C/N ratio is higher than bacteria (Paul and Clark, 1989). Beginning at two weeks, some groupings were formed and maintained: (SR, SL, CR, CL), (RCL, TL), (RCR), (TR, Soil).

5.3.3 Relationship between initial plant material quality and microbial community composition

The relationship between FAMEs and plant material quality indicators was studied through RDA analysis in an attempt to identify the quality indicators explaining the variations in FAME profiles (Figure 5.3). The incubated materials had different chemical characteristics (Table 5.2). The RDA analysis was performed with FAME data collected at 3 days, 1, 2, 7, 13, 16 and 28 weeks after the beginning of incubation. However, the Monte Carlo permutation test demonstrated that the relation between initial plant material quality and FAME profiles was strong and significant only at 1, 7 and 13 weeks of incubation ($P = 0.005$ for both the first and all canonical axes).

5.3.3.1 After one week of incubation

After one week of incubation (Figure 5.3a), the initial percentages of hemicellulose and fiber in the incubated materials were most important at influencing soil FAME profiles ($P = 0.005$), explaining 37.7 and 34% of the total explained variation, respectively. Because % fiber and % soluble C are highly negatively correlated, only % fiber was included in the RDA analysis. The effect of % fiber on FAME variation as early as one week after the beginning of the incubation could, in fact, be attributed to the highly correlated soluble C fraction of the incubated material.

One week after the onset of the incubation, correlation analyses with individual fatty acids (Table 5.3) showed initial % fiber of plant material was negatively correlated with anteiso Gram(+) bacteria fatty acids a15:0 and a17:0 and positively correlated with iso Gram(+) bacteria fatty acids i15:0 and i17:0. This suggests that higher % fiber in incubated material increases with the iso/anteiso ratio of these fatty acids. A decrease in the iso/anteiso ratio was associated to increased C availability by McKinley et al. (2005), McMahon et al. (2005) and Haack et al. (1994). Similarly, cyclopropyl Gram(-) bacteria fatty acid cy19:0 was positively correlated with initial % fiber while its precursor fatty acid 18:1w7c was negatively correlated. This suggests that the cyclo/precursor ratio of this fatty acid increases with the % fiber of incubated material. This ratio has been related to the nutritional status of Gram(-) bacteria and was shown to increase with decreasing C availability and nutritional imbalance (Tunlid et al., 1985; Guckert et al., 1986; Bossio and Scow, 1995; Phillips et al., 2002). The

initial % fiber of incubated material was only negatively correlated with Gram(-) bacteria; no significant correlation was found with Gram(+) bacteria (Table 5.4). This suggests that the proportion of Gram(-) bacteria decreases when incubated material has higher fiber content or lower content of soluble C (% soluble C= 100-% fiber). Recent studies have shown Gram(-) bacteria to preferentially use recently added carbon sources while Gram(+) bacteria use older soil organic matter (Kramer and Gleixner, 2006; Waldrop and Firestone, 2004). Gram(-) bacteria might thus be more responsive to readily available C sources that can be decomposed faster while Gram(+) bacteria, considered to be slow growing, tend to be able to degrade more complex compounds (Paul and Clark, 1989). This might explain why, at one week after the beginning of incubation, the % fiber (or % soluble C) affects the nutritional status or physiological state of both Gram(-) and Gram(+) bacteria while affecting the proportion of only Gram(-) bacteria.

Percentage of hemicellulose was only related to some of the branched Gram(+) bacteria fatty acids and with one of the cyclopropyl Gram(-) bacteria fatty acid (Table 5.3), and, overall, was negatively correlated with Gram(+) bacteria (Table 5.4). There is no evidence in the literature that Gram(+) bacteria are not able to use hemicellulose as a carbon source nor that they are negatively affected by hemicellulose.

Percent lignin was also important (P = 0.02) after 1 week of incubation, and explained 15.1% of the total explained variation in soil FAME profiles (Figure 5.3a). Initial %lignin of plant material was positively correlated with iso Gram(+) bacteria fatty acids i15:0 and i17:0 as well as cyclopropyl Gram(-) bacteria fatty acids cy17:0 and cy19:0 (Table 5.3). Although no correlations were found with anteiso and precursors fatty acids (a15:0, a17:0, 16:1ω7c and 18:1ω7c), this suggests that, at this point in the incubation, iso/anteiso and cyclo/precursor ratios and thus nutritional status of both Gram(+) and Gram(-) bacteria might be negatively affected by the %lignin. The initial %lignin was not correlated with proportions of Gram(-) or Gram(+) bacteria (Table 5.4) suggesting that lignin might only affect the nutritional status of bacteria. Since lignin is relatively resistant to decomposition, at the beginning of incubation bacteria will most likely utilize more easily decomposable compounds such as cellulose and hemicellulose. However, lignin encrustation of cellulose and hemicellulose protect them from microbial attack and some degree of lignolysis is therefore necessary to expose these structural polysaccharides to bacterial decomposition.

The initial %lignin was also positively correlated with actinomycete markers (10Me16:0 and 10Me18:0) and proportions while negatively correlated with fungal markers (18:1ω9c and 18:2ω6,9c) and proportions of fungi (Tables 5.3 and 5.4). Both fungi and actinomycetes are recognised as being able to decompose lignin (e.g., McCarthy, 1987; Kirk and Farrell, 1987), however, there is evidence that actinomycetes can decompose lignin during normal growth while decomposition by fungi is induced by starvation (McCarthy and Broda 1984). Lignin degradation by fungi has been shown to happen only during secondary metabolism and to be induced by the depletion of nitrogen, carbon, or sulphur (Jeffries et al, 1981; Kirk and Farrell, 1987; Aust, 1990). After only one week of incubation, fungi might be using compounds other than lignin since the conditions inducing lignin degradation by fungi would not have occurred. Nitrogen addition at the beginning of the incubation might also have affected fungal capacity to degrade lignin as it has been shown that fungal synthesis of ligninolytic enzymes can be inhibited or delayed by ammonium addition (Keyser et al., 1978).

5.3.3.2 After seven weeks of incubation

After 7 weeks of incubation, the initial C/N ratio and the lignin content of incubated materials affected the soil FAME composition (P = 0.005 and 0.035, respectively) and explained 46.1 and 15.4% of the total explained variation respectively (Figure 5.3b).

The C/N ratio of decomposing material has been shown to affect C mineralization during the early stage of decomposition. In this study, the C/N ratio had no effect on microbial composition until after 7 weeks of incubation, probably because N was added at the beginning of the incubation. After 7 weeks, the soil is probably N depleted and microorganisms become dependent on N provided by the added organic material. Fungal markers 18:2ω6,9c and 18:1ω9c were positively correlated while all bacterial markers showed a negative correlation with initial C/N ratio of incubated materials (Table 5.3). The C/N ratio was negatively correlated with proportions of actinomycetes, Gram(+) and Gram(-) bacteria, and positively correlated with proportions of fungi (Table 5.4). Materials with high C/N ratios shifted the composition of the microbial community toward increased proportions of fungi. The lower N requirement of fungi as compared to bacteria (Paul and Clark, 1989) and their capacity to adjust the C/N ratio of their mycelium according to the C/N ratio of available substrate (e.g.,

94

Levi and Cowling, 1969) might favour their growth over bacteria in presence of higher C/N ratio materials.

The correlations between % lignin and individual fatty acids were similar to the ones obtained after 1 week of incubation, however, no correlations were found with any of the groups of microorganisms suggesting that lignin might affect the physiological status of the microbial community rather than its composition.

5.3.3.3 After thirteen weeks of incubation

After 13 weeks of incubation, the initial %fiber and the lignin content of incubated material had the greatest effect on soil FAME profiles and explained 50 and 20% of the total explainable variation, respectively (P=0.005 and 0.035, respectively, Figure 5.3c).

At 13 weeks of incubation, fatty acids and microbial groups correlations with lignin content of incubated plant materials were similar to the correlations observed at 7 weeks.

By this time, the more easily decomposable compounds of plant material were likely used up by the soil microorganisms and only the more recalcitrant compounds remained. Most bacterial fatty acids, including Gram(-) and Gram(+) markers, as well as bacterial fatty acids related to physiological/nutritional status of bacteria, were negatively correlated with %fiber of incubated material (Table 5.3). The %fiber was also negatively correlated with the proportion of Gram(+) and Gram(-) bacterial FAMEs. This suggests that, compared with the effect of fiber at 1 week of incubation, after 13 weeks, the fiber content of incubated material strongly affected the bacterial (both Gram(-) and Gram(+)) composition of the microbial community. Increased proportion of fiber in incubated material positively affected the proportion of fungi while negatively affecting the proportion of bacteria and actinomycetes. Although actinomycetes and bacteria are able to efficiently degrade free cellulose and hemicellulose (due to the lack of powerful fiber degrading enzymes, especially lignin peroxidase), fiber degradation by bacteria and actinomycetes is a slow process (Malherbe and Cloete, 2001). Fungi, on the other hand, are widely recognised for their ability to efficiently degrade fiber (e.g., Ericksson et al., 1990; Malherbe and Cloete, 2002).

After 13 weeks, there was no significant relationship between initial material quality and the structure of the microbial community. At this point in the decomposition process, incubated

materials have probably been modified by microbial degradation to such an extent that their initial chemical characteristics no longer determine the structure of the microbial community.

5.4 CONCLUSION

This experiment showed that shifts in FAME profiles of the soil microbial community during incubation with plant materials were different depending on the incubated material. Various chemical characteristics of the added material appeared to influence the composition of the microbial community at different times during the decomposition process. The percentages of soluble C and hemicellulose affected the microbial community composition at the early stage of decomposition, increasing Gram(-) and decreasing Gram(+) bacteria proportions, respectively. The C/N ratio and the % fiber exerted their effect later in the process, increasing the fungi-to-bacteria ratio while the % lignin affected the nutritional or physiological status of bacteria without affecting their relative abundance.

Indications that the composition of the microbial community might affect soil aggregation, and the presumption that fungi dominated communities may favour soil carbon accumulation because fungi use carbon more efficiently and are more resistant to decay than bacteria, suggest that the composition of the microbial community might be important when carbon storage in soil is a concern. Understanding how residue chemistry influences microbial community composition and activity may therefore have implications in research areas such as soil carbon storage.

Further investigations considering other variables that also influence the composition of the microbial community (e.g., moisture, temperature, tillage practice) are necessary in order to determine the importance of the chemical quality of decomposing material in influencing the composition of the microbial community in field situations.

Table 5.1: Fatty acid methyl esters (FAME) extracted from incubated soils.

Fatty acid	Marker
14:0	Not specific
15:0	Bacteria[e]
16:0	Not specific
17:0	Bacteria[e]
18:0	Not specific
20:0	Not specific
i-14:0	Gram(+)[b]
i-15:0	Gram(+)[c]
i-16:0	Gram(+)[c]
i-17:0	Gram(+)[c]
a-15:0	Gram(+)[c]
a-17:0	Gram(+)[c]
16:1ω11c	Gram(-)[b]
16:1ω7c	Gram(-)[c]
16:1ω5c	Mycorrhizal Fungi[a]
17:1ω8c	
18:1ω9c	Fungi[b,c]
18:1ω7c	Gram(-)[c]
18:1ω5c	Gram(-)[c]
20:1ω9c	Fungi [a]
18:2ω6,9c	Fungi[a,c]
Cy17:0	Gram(-)[c]
Cy19:0	Gram(-)[c]
10Me16:0	Gram(+)[c], Actinomycetes[d]
10Me17:0	Actinomycetes[e]
10Me18:0	Actinomycetes[d]
16:1-2OH	
17:0-2OH	
18:0-2OH	

[a]Madan et al., 2002; [b]Zelles1997; [c]Zak et al. 1996; [d]White et al., 1997; [e]Ritchie et al., 2000

Table 5.2: Initial chemical characteristics of incubated plant materials

Material	C/N	%hemicellulose	%fiber	%lignin	lignin/N
Corn leaves+stems	72a[†]	33a	81a	6f	11c
Corn roots	43c	31b	77a	11de	10cd
Red clover leaves+stems	17fg	10f	47de	15c	7de
Red clover roots	14g	13[e]	43e	13cd	5ef
Soybean leaves+stems	48b	16d	63c	10e	11c
Soybean roots	34d	17d	79a	20b	16b
Timothy leaves	20f	21c	49d	6f	3f
Timothy roots	26e	20c	68b	29a	24a

[†]Numbers within a column followed by the same letter(s) are not significantly different at the 0.05 probability level.

Table 5.3: Pearson correlation coefficients (r values) between fatty acid proportions (% total FAME) and plant material chemical characteristics (n=24).

| | Time since plant material addition to soil | | | | | | |
| | 1week | | | 7 weeks | | 13 weeks | |
Fatty acid	%Fiber	%Hemi-cellulose	%Lignin	C/N	%Lignin	%Fiber	%Lignin
i-14:0	Ns	-0.53**	0.43*	-0.86***	0.58**	-0.72***	0.48*
14:0	Ns	ns	ns	-0.77***	0.50*	-0.74***	ns
i-15:0	0.42*	ns	0.62**	-0.80***	0.73***	-0.55**	0.65***
a-15:0	-0.87***	-0.83***	ns	-0.90***	ns	-0.90***	ns
15:0	Ns	ns	0.62**	-0.71***	0.57**	-0.59**	0.43*
i-16:0	-0.60**	-0.78	ns	-0.85***	ns	-0.82***	ns
16:1ω11c	Ns	ns	ns	-0.61**	0.49*	ns	ns
16:1ω7c	-0.59**	ns	ns	-0.78***	ns	-0.72***	ns
16:1ω5c	Ns	ns	ns	-0.52**	0.67***	ns	0.73***
16:0	0.66***	ns	ns	ns	0.47*	ns	0.80***
10Me16:0	0.43*	ns	0.46*	-0.66***	0.68***	ns	0.68***
i-17:0	0.56**	ns	0.50*	-0.84***	0.57**	-0.54**	0.51*
a-17:0	-0.63***	-0.79***	ns	-0.92***	ns	-0.80***	ns
17:1ω8c	-0.62**	ns	ns	-0.85***	ns	-0.90***	ns
Cy17:0	Ns	-0.57**	0.70***	-0.89***	0.53**	-0.72***	0.51*
17:0	Ns	-0.45*	0.44*	ns	0.43*	-0.68***	ns
16:1-2OH	0.69***	ns	0.59**	-0.75***	0.78***	ns	0.72***
10Me17:0	Ns	ns	ns	-0.54**	ns	-0.59**	ns
18:2ω6,9c	Ns	ns	-0.74***	0.84***	-0.48*	0.66***	-0.47*
18:1ω9c	-0.52**	ns	-0.52**	0.43*	-0.62**	ns	-0.45*
18:1ω7c	-0.40*	ns	ns	-0.71***	0.58**	-0.45*	0.67***
18:1ω5c	-0.62**	ns	ns	-0.61**	ns	-0.65***	ns
18:0	Ns	ns	ns	ns	0.58**	ns	0.84***
17:0-2OH	0.55**	ns	ns	ns	ns	ns	ns
10Me18:0	0.48*	ns	0.52**	-0.77***	ns	-0.69***	ns
Cy19:0	0.68***	ns	0.67***	-0.75***	0.66***	-0.50*	0.63**
18:0-2OH	Ns	ns	ns	-0.47*	ns	ns	ns
20:1ω9c	0.43*	ns	ns	ns	0.45*	0.58**	ns
20:0	0.59**	ns	0.51*	-0.55**	0.85***	ns	0.79***

ns : not significant; *: 0.01<P<0.05; **: 0.001<P<0.01; ***: P<0.001

Table 5.4: Pearson correlation coefficients (r values)   between proportions of microorganisms (%total FAME) and plant material chemical characteristics (n=24)

| Microorganisms | 1week | | | 7 weeks | | 13 weeks | |
	%Fiber	%Hemi-cellulose	%Lignin	C/N	%Lignin	%Fiber	%Lignin
Gram(-) bacteria	-0.60**	ns	ns	-0.84***	ns	-0.73***	ns
Gram(+) bacteria	ns	-0.64***	ns	-0.92***	ns	-0.77***	ns
Actinomycetes	ns	ns	0.62**	-0.73***	ns	-0.50*	ns
Fungi	ns	ns	-0.54**	0.84***	ns	0.65***	ns

ns : not significant; *: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$; ***: $P < 0.001$

Figure 5.1: Principal component analysis (PCA) of community FAME profiles throughout the incubation period for the different applied treatments. Twenty nine fatty acids (Table 5.1) were used for the bi-plots. a) CR, corn roots; b) CL, corn (leaves+stems); c) SR, soybean roots; d) SL, soybean (leaves+stems); e) RCR, red clover roots; f) RCL, red clover (leaves+stems); g) TR, timothy roots; h) TL timothy (leaves+stems); i) Soil, no material addition. Percentages represent the amount of variability explained by the principal component.

Figure 5.2: Principal component analysis (PCA) of community FAME profiles of soil incubated with different organic amendments at the beginning of incubation (a), after 3 days (b), and after 1, 2, 7, 13, 16 and 28 weeks (c, d, e, f, g and h respectively). Twenty nine fatty acids (Table 5.1) were used for the bi-plots. CL, corn (leaves+stems); CR, corn roots; SL, soybean (leaves+stems); SR, soybean roots; RCL, red clover (leaves+stems); RCR, red clover roots; TL timothy (leaves+stems); TR, timothy roots; Soil, no material addition. Percentages represent the amount of variability explained by the principal component.

Figure 5.3: Redundancy analysis (RDA) exploring the relationship between initial plant material quality and FAME profile at 1 (a), 7 (b) and 13 (c) weeks after plant materials addition to soil. Chemical characteristics of the plant materials are given in Table 5.2; these were used as explanatory variables. The 29 FAME given as species variables are listed in Table 5.1; for the sake of clarity, some FAME have not been identified on the ordination diagram. Percentages represent the amount of variability explained by the axis.

REFERENCES

Allison V. J., Miller R. M., Jastrow J. D., Matamala R., and. Zak D. R. 2005. Changes in Soil Microbial Community Structure in a Tallgrass Prairie Chronosequence. Soil Science Society of America Journal 69:1412–1421

Aneja, M.K., S. Sharma, J.C. Munch, and M. Schloter. 2004. RNA fingerprinting—a new method to screen for differences in plant litter degrading microbial communities. Journal of Microbiological Methods 59:223–231.

Aust, S.D. 1990. Degradation of environmental pollutants by Phanerochaete chrysosporium. Microbial Ecology 20(2) p. 197-209

Bardgett, R.D., Lovell R.D., Hobbs P.J., Jarvis S.C. 1999. Seasonal changes in microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry 31: 1021-1030

Berg, B. and McClaugherty, C. 2003.Decomposer Organisms. In: Plant litter: decomposition, humus formation, carbon sequestration. Edited by Berg, B. and McClaugherty, C. Springer-Verlag, Berlin Heidelberg, Germany.

Bossio, D.A. and Scow, K.M. 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. Applied Environmental Microbiology 61:4043–4050

Broder, M.W., and G.H. Wagner. 1988. Microbial colonization and decomposition of corn, wheat, and soybean residue. Soil Science Society of America Journal 52:112–117.

Buyer, J. 2002. Identification of bacteria from single colonies by fatty acid analysis. Journal of Microbiological Methods 48:259-265.

Calderon F.J., Jackson L.E., Schow K.M., Rolston D.E. 2001. Short-term dynamics of nitrogen, microbial activity and phospholipids fatty acids after tillage. Soil Science Society of America Journal 65, 118-126.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology and Biochemistry 32, 1419-1430.

Ericksson K- E, RA Blanchette and P Ander. 1990. In: Timell TE (Ed.), Microbial and Enzymatic Degradation of Wood and Wood Components. Springer -Verlag, Berlin, pp. 1 –407.

Guckert, J.B., Hood, M.A. and White, D.C. 1986. Phospholipid, ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increase in the *trans/cis* ratio and proportions of the cyclopropyl fatty acids. Applied Environmental Microbiology 52:794-801.

Haack, S.K., Garchow, H., Odelson, D.A., Forney, L.J. and Klug, M.J. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied Environmental Microbiology 60:2483–2493

Heal, O.W., J.M. Anderson and M.J.Swift. 1997. Plant litter quality and decomposition: An historical overview. In: Driven by nature: plant litter quality and decomposition. Edited by G. Cadisch and K.E. Giller. CAB INTERNATIONAL, Wallingford, UK.

Henriksen, T.M., and T.A. Breland. 2002. Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil. Biology and Fertility of Soils 35:41–48.

Ibekwe A.M. and Kennedy A.C. 1999. Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant and Soil, 206:151-161

Jeffries, T. W., Choi, S. and Kirk, T.K. 1990. Nutritional Regulation of Lignin Degradation by *Phanerochaete chrysosporium*. Applied Environmental Microbiology 1981 42: 290-296.

Keyser, P., Kirk, T.K., and Zeikus, J.G. 1978. Ligninolytic enzyme system of Phanerochaete chrysosporium: synthesis in the absence of lignin in response to nitrogen starvation. Journal of bacteriology 135:790-797.

Kirk, T. K. and Farrell, R. L. 1987. Enzymatic "combustion": the microbial degradation of lignin. Annual Review of Microbiology, 41: 465

Klug M J and Tiedje J M. 1993. Response of microbial communities to changing environmental conditions: chemical and physiological approaches. *In* Trends in Microbial Ecology. Eds. R Guerrero and C Pedros-Alio (eds). Pp 371-374. Spanish Society for Microbiology, Bacelona, Spain.

Knivett VA, Cullen J. 1965. Some factors affecting Cyclopropane acid formation in *Escherichia coli*. Biochemical Journal; 96:771–776

Kramer, C. and Gleixner, G. 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. Soil Biology Biochemistry 38: 3267-3278.

Levi, M.P. and Cowling, E.B. 1969. Role of nitrogen in wood deterioration: VII. Physiological adaptation of wood destroying and other fungi to substrates deficient in nitrogen. Phytopathology 59:460-468.

Lundquist,E.J., L.E. Jackson, K.M. Scow, and C. Hsu. 1999. Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology and Biochemistry 31:221–236.

Madan, R., Pankhurst, C., Hawke, B. and Smith, S. 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. Soil Biology and Biochemistry 34: 125-128.

Malherbe, S. and Cloete, T.E. 2002. Lignocellulose biodegradation: Fundamentals and applications. Reviews in Environmental Science and Biotechnology 1: 105–114.

McCarthy A J, Broda P (1984) Screening for lignin-degrading actinomycetes and characterization of their activity against (^{14}C)lignin-labelled wheat lignocellulose. Journal of General Microbiology 130:2905-2913

McCarthy, A. J. 1987. Lignocellulose-degrading actinomycetes. FEMS Microbiology Review 46:145-163

McKinley, V.L., Peacock, A.D. and White, D.C. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairies soils. Soil Biology and Biochemistry 37: 1946-1958.

McMahon, S.K., Williams, M.A., Bottomley, P.J. and Myrold, D.D. 2005. Dynamics of Microbial Communities during Decomposition of Carbon-13 Labeled Ryegrass Fractions in Soil. Soil Science Society of America Journal 69:1238-1247

Molope, M.B. and Page, E.R. 1986. The contribution of fungi, bacteria and physical processes in the development of aggregate stability of a cultivated soil. Biological Agriculture and Horticulture 3: 233-249.

Myers, R.T., D.R. Zak, D.C. White, and A. Peacock. 2001. Landscape- level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65:359–367.

Nakamura, A., C.C. Tun, S. Asakawa, and M. Kimura. 2003. Microbial community responsible for the decomposition of rice straw in a paddy field: Estimation by phospholipids fatty acid analysis. Biology and Fertility of Soils 38:288–295.

Neely, C.L., M.H. Beare, W.L. Hargrove, and D.C. Coleman. 1991. Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. Soil Biology and Biochemistry 23:947–954.

Oades, J. M. and Waters, A.G. 1991. Aggregate hierarchy in soils. Australian Journal of Soil Research 29: 815-828.

Pankhurst C.E. 1997. Biodiversity of soil organisms as an indicator of soil health. *In* Biological indicators of soil health. Eds. C.E. Pankhurst, B.M. Doube and V V S R Gupta. Pp 297-324. New York, NY.

Paul, E.A., and F.E. Clark. 1996. Soil microbiology and biochemistry. 2nd ed. Academic Press, San Diego, CA.

Phillips, R.L., Zak, D.R., Holmes, W.E. and White D.C. 2002. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131: 236-244

Ritchie, N. J., Schutter, M.E., Dick, R.P. and Myrold, D.D. 2000. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied and Environmental Microbiology 66: 1668-1675.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Tech. Note #101. Microbial ID, Newark, USA.

Schimel, J. 1995. Ecosystem consequences of microbial diversity and community structure. p. 239–254. *In* F. S. Chapin, III and C. Körner (ed.) Ecological Studies v. 113: Arctic and Alpine Biodiversity. Springer-Verlag, Berlin.

Schutter, M. and Dick, R. 2001. Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biology and Biochemistry 33: 1481-1491.

Sharma, S. A., Rangger, A. and Islam, H. 1998. Effect of decomposing maize litter on community level physiological profiles of soil bacteria. Microbial Ecology 35: 301-310.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in terrestrial ecosystems. Blackwell, Oxford, UK.

Thomas TD, Batt RD (1969) Degradation of cell constituents by starved *Streptococcus lactis* in relation to survival. Journal of General Microbiology 58:347–362.

Tisdall, J.M., Smith, S.E., Rengasamy,P. 1997. Aggregation of soil by fungal hyphae. Australian Journal of Soil Research 35: 55-60.

Tunlid, A., Baird, B. H., Trexler, M.B., Olsson, S., Findlay, R.H., Odham, G. and White, D.C. 1985. Determination of phospholipids ester-linked fatty acids and poly beta hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* (L). Canadian Journal of Microbiology 31:1113-1119.

Van Soest P.J. and Wine R.H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. Journal of the Association of Official Analytical Chemists 50: 50-55.

Van Soest P.J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Journal of the Association of Official Analytical Chemists 46: 828-835.

Waldrop, M.P. and Firestone M.K. 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138: 275–284

Wardle, D.A. 1995. Impact of disturbance on detritus food-webs in agroecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26:105-185.

White, D.C., Stair, J.O. and Ringelberg, D.B. 1997. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. Journal of Industrial Microbiology 17: 185-196.

Zak, D.R. Ringelberg, D.B., Pregitzer, K.S., Randlett, D.L., White, D.C. and Curtis, P.S. 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecological Applications 6: 257-262.

Zelles, L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35: 275-294.

Zelles, L., Q.Y. Bai, T. Beck, F. Beese. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbiological biomass and community structure in agricultural soils. Soil Biology and Biochemistry 24: 317-323.

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

CHAPTER 6

Summary and General Conclusions

6.1 Review of research objective

The main objective of this research was to understand the fate of carbon under different cropping systems emphasizing the implications of the soil microbial community and its effect on soil structure. The rationale of this research was that the relationship between the size of the microbial community and soil structural stability has not yet been clearly identified and the relationships between the composition of the microbial community and soil structural stability have only been poorly explored. A good understanding of the process of aggregation and the factors by which soil structure is influenced is important if we want to adopt management practices that will be effective in increasing or at least maintaining soil carbon. Soil microorganisms play a key role when it comes to carbon storage in soil since they are responsible for organic matter decomposition but also take part in the processes of chemical stabilization and physical protection of the organic matter through their impact on soil structural stability. A three-year field study was conducted to assess the effects of different cropping systems on soil structure and carbon levels, to explore the relationship between soil structure and microbial community structure, and to determine the cropping system characteristics that were the most important in influencing the composition of the microbial community.

In the field, the microbial community and the process of aggregation are influenced by many factors including tillage, crop biomass, crop roots, residue quality and climate. An incubation experiment was conducted to isolate the effect of organic matter quality on microbial community and soil structure and to explore the relationship between initial residue quality and microbial community on the one hand and between microbial community structure and soil structural stability on the other hand.

6.2 Main findings

Corn, sorghum, soybean, red clover and timothy were produced on the same plots for three consecutive years in a multi-crop field experiment and using the appropriate tillage and

crop residue managements for each crop. The studied cropping systems differed in the level of tillage, in root morphology, and in nature and amount of organic input to soil. The microbial community composition was studied using fatty acid methyl ester (FAME) analysis providing information about the size, the composition and the nutritional status of the microorganisms.

The cropping systems affected soil structure, carbon content and microorganisms differently. After three growing seasons, the contrasting cropping systems involving sorghum and timothy on the one hand, and corn and bare fallow on the other hand, had similar effects on microbial activity, soil structure and total carbon content. Surface-tillage was used in sorghum and biomass input to soil was eight times greater in sorghum than timothy but the latter had longer periods of active growth, a finer and more extensive root system and was not tilled for three years. Surface tillage was used in both corn and bare fallow, but while there was essentially no carbon input to soil in the latter, 40 t/ha of corn residues were returned to the field over the three years.

Soil total carbon content was increased only under the two perennial crops, as compared to bare fallow. Plant root systems seemingly have a very large influence on soil processes. Perennial crop effects on soil carbon processes were exerted exclusively through their roots as all above-ground biomass was exported. The relatively short duration of the study certainly contributed to the fact that the other cropping systems had no detectable effect on soil carbon. Indeed, soil carbon changes slowly and after 3 years, some changes were probably too small to be detected against the large background of soil carbon.

A relationship between microbial community structure and soil structure was indicated by correlations found between some FAME groups and soil structure. As expected, AMF and fungi in general were associated with larger MWD and proportion of macroaggregates. Interestingly, FAME groups associated with Gram(-) bacteria also appeared positively related to larger MWD and proportion of macroaggregates, while Gram(+) bacteria showed opposite correlations with soil structure variables. This suggested that Gram(-) contribute to soil macroaggregation, whereas Gram(+) bacteria do not.

The relative importance of soil management practices and crop characteristics as factors affecting the microbial community composition was assessed using tillage practice, carbon input to soil, roots morphology, roots and above-ground material chemical quality and biomass as the cropping systems' descriptors. Microbial community structure was mainly related to root

biomass and initial lignin content. The relationship between residue quality and soil microbial community structure was further explored during crop residue decomposition. Lignin content influenced soil microbial community structure during the first three months of decomposition and seemed to affect the nutritional or physiological status of bacteria rather than their proportions. Soluble carbon, hemicellulose and fiber content, as well as C/N ratio of decomposing residues, were other initial chemical properties affecting soil microbial community structure. High soluble C and hemicellulose content increased the proportion of Gram(-) bacteria and decreased the proportions of Gram(+) bacteria, respectively, while high C/N and % fiber both increased the fungi-to-bacteria ratio.

Interestingly, soil structure was best promoted during decomposition of soybean residues, which had intermediate quality value, but no improvement in soil structure was found in soybean planted field soil, pointing out the importance of interacting variables and their effect on soil processes. Tillage, small root and shoot residues input to soil and the shallow taproot system might all have contributed to the low MWD and % macroaggregates observed under soybean in the field.

The relationship between microbial community structure and soil structure found in the field was further explored in the laboratory where plant materials amended soil was incubated for 28 weeks. Changes in microbial community structure resulted in change in soil structure components. Proportion of macroaggregates and MWD were increased by fungi and Gram(-) bacteria, supporting field observations. The relation between fungi and macroaggregates was an established fact but Gram(-) bacteria had never been observed to be associated with macroaggregates in previous published studies. Differences in life strategy between Gram(-) and Gram(+) bacteria were proposed as a possible explanation for this correlation. The organic carbon in macroaggregates is younger and less decomposed than the organic carbon in microaggregates and Gram(-) bacteria develop preferentially on fresh, newly added substrates. Gram(-) bacteria are more abundant and metabollicaly active than Gram(+) bacteria in the rhizosphere, suggesting that apart from their effect on soil physical enmeshment, root systems could also improve soil structure indirectly through rhizospheric microorganisms, in particular Gram(-) bacteria.

6.3 Conclusion

This study showed that carbon storage should be considered in a system wide perspective because both high and low carbon storage can be arrived at through a range of conditions, and can involve different balances between different sets of variables. The importance of carbon storage in soil is not only a function of crop biomass input, as it also depends on soil processes, as affected by cropping systems. The effects of the root system, nature and amount of carbon inputs and soil management practices on soil aggregation, microorganisms and organic matter decomposition are all important to consider. The aggregating capacity of Gram(-) versus Gram(+) bacteria and other groups of microorganisms (e.g., actinomycetes, bacterial and fungal species) is an area that merits further investigation. Understanding how residue chemistry affects the soil microbial community and identifying the relationships between cropping system, microbial community structure and soil structure might give us the possibility to predict the fate of, and better manage the soil carbon pool. This pool is the cornerstone of soil quality and a key regulator of the Earth's chemical equilibrium.

Contributions to the advancement of knowledge

The work presented in this thesis contributed to the advancement of scientific knowledge in several ways:

1) Chapters 3 and 4 present the first studies suggesting different implications of Gram(-) and Gram(+) bacteria in soil aggregation.

The fact that relations between microbial community structure and soil structure were consistent in the field experiment and in the laboratory study suggests that these relationships exist in other situations. More experiments are required to further explore the prevalence of those previously unknown relationships. Better understanding of the microbial community structure associated with different size classes of aggregates will contribute to the advancement of knowledge and understanding of the implication of soil microorganisms in the process of building and stabilization of soil structure.

2) Lignin is usually considered to influence the later stage of decomposition. Chapter 5 presents the first study suggesting that lignin content of decomposing material affects the nutritional status of the microbial community in the early stage of decomposition. This contributes to the advancement of knowledge and understanding of the process of organic matter decomposition by soil microorganisms. Better knowledge of the influence of crop residue chemical characteristics on microbial community on the one hand and of the microbial community effects on soil structure on the other hand is essential for the understanding of the impact of crop residue decomposition on soil aggregation.

3) This research has shown soil structure to be affected by the composition of the soil microbial community. Chapters 3 and 4 present two of the rare studies of the relationship between soil structure and soil microbial community composition. Most published studies have related soil structure to the relative importance of bacterial and fungal biomass or to the total microbial community size and/or activity. The multi-cropping system field-based experiment provided the opportunity to study this relationship in a context where crop species, quality and quantity of carbon input to soil as well as soil management practices (surface tillage or no

tillage) might all have affected the composition of the microbial community and soil aggregation.

4) To my knowledge, my incubation experiment is the first study relating the quality of plant residue input to soil to soil microbial community structure rather than activity. Chapter 5 presents the first study showing that soil microbial community FAME composition is differentially affected by the decomposition of crop material with different chemical characteristics

5) Overall, findings of these field and laboratory trials will contribute to building the scientific basis for understanding the role of soil microorganisms in soil carbon storage through their implications in organic matter decomposition and protection within soil aggregates.

Appendices
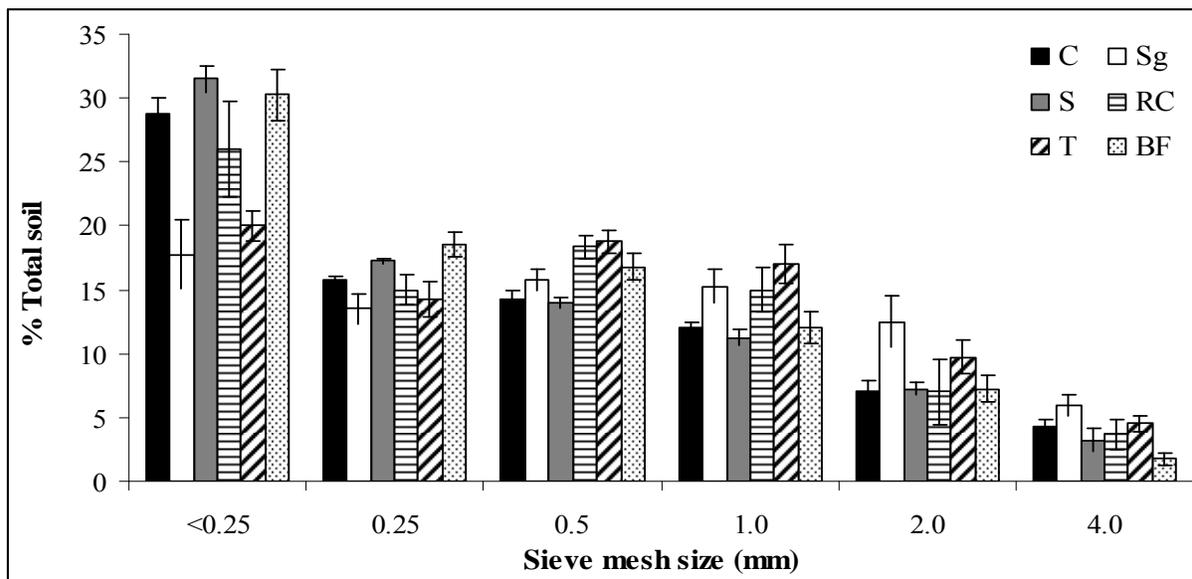
Figure A.1: Dry aggregates size distribution under the different cropping systems after three growing seasons. The cropping systems referred to as C, Sg, S, RC, T and BF involve corn, sorghum, soybean, red clover, timothy and bare fallow respectively. The vertical bars represent 2 x SE mean (n=4).
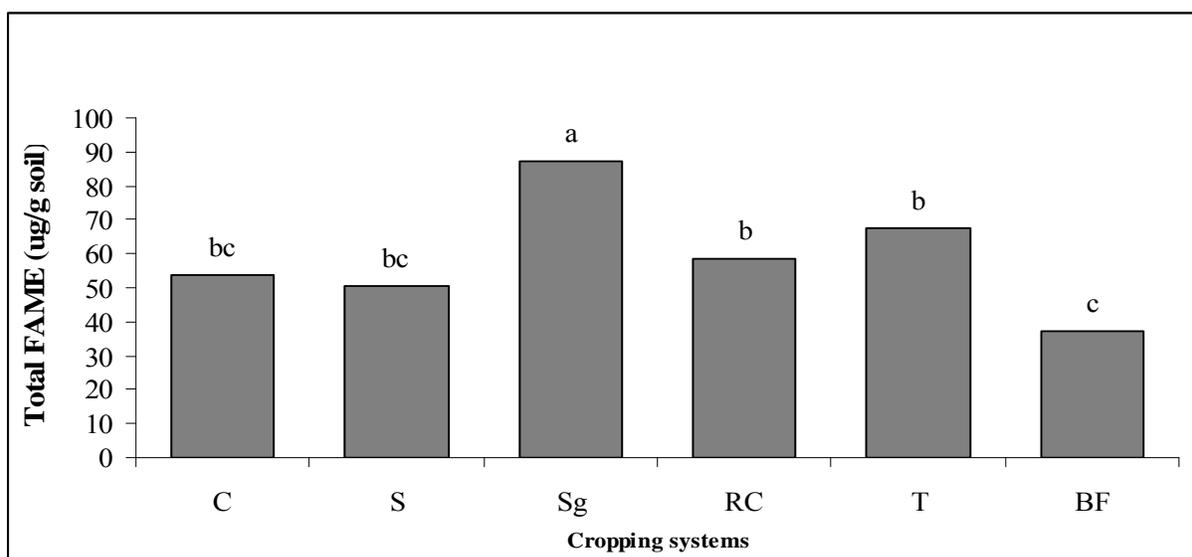
Figure A.2: Total fatty acid methyl ester (FAME) extracted from soil under the different cropping systems after 3 growing seasons. The cropping systems referred to as C, Sg, S, RC, T and BF involve corn, sorghum, soybean, red clover, timothy and bare fallow respectively. FAME values followed by the same letter are not significantly different (P=0.05) according to Bonferroni (n=4).
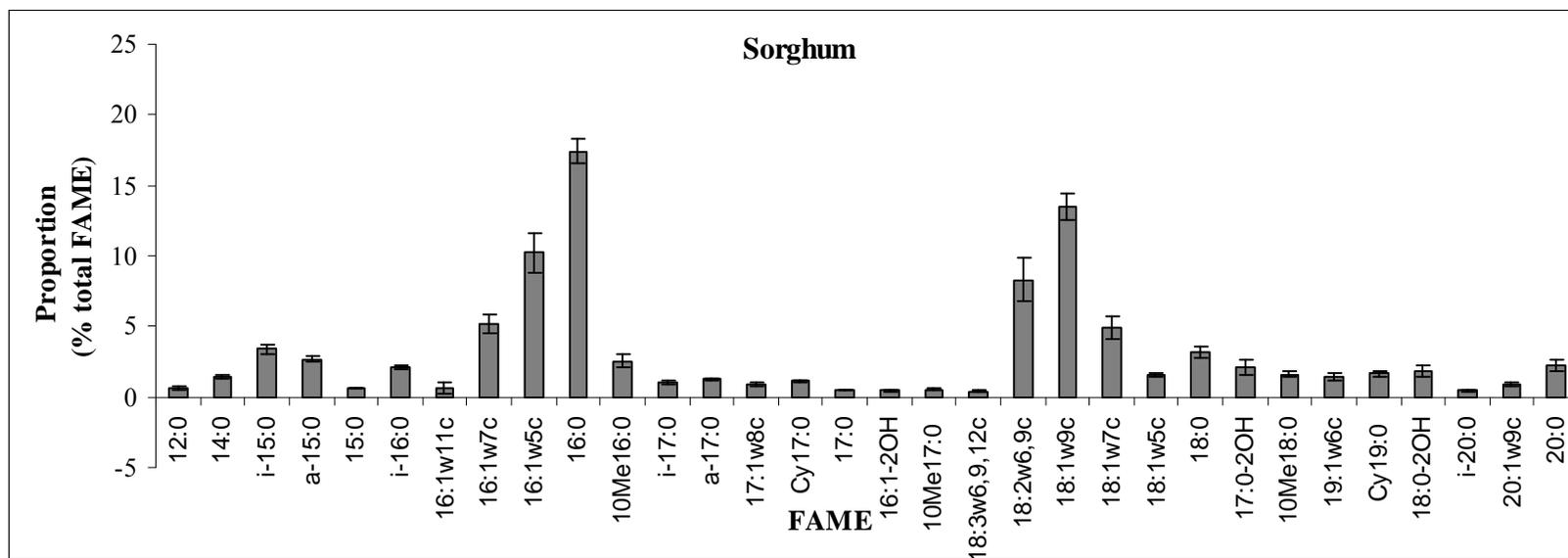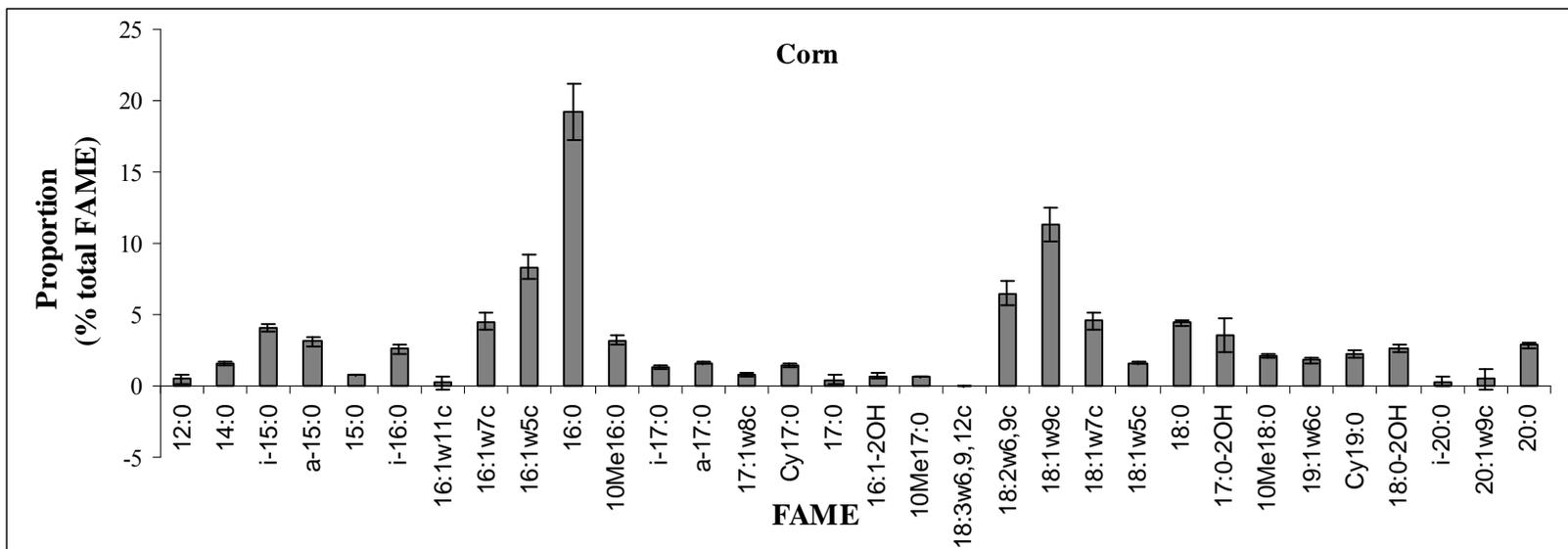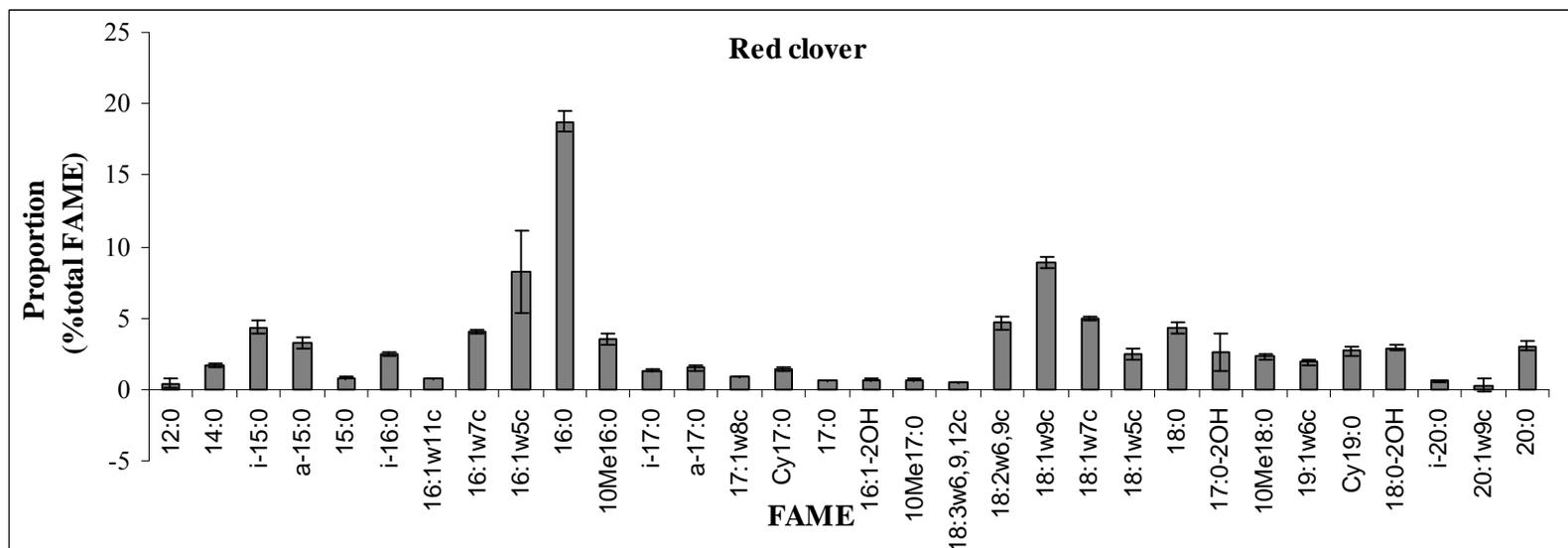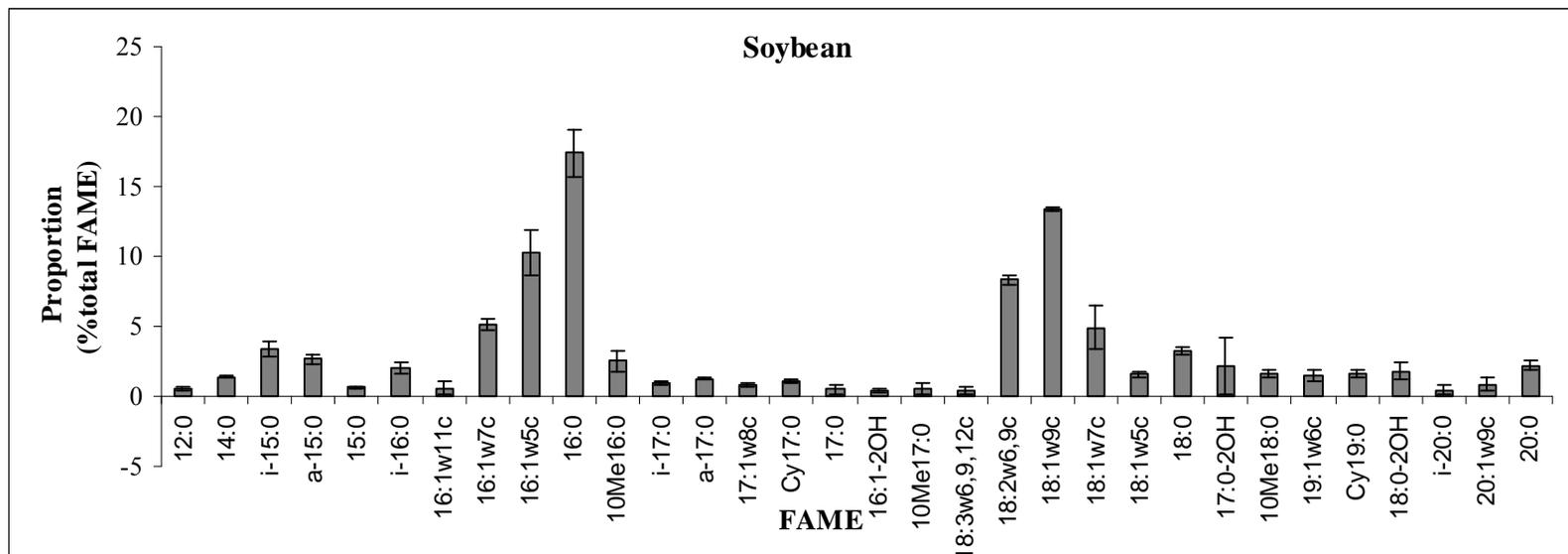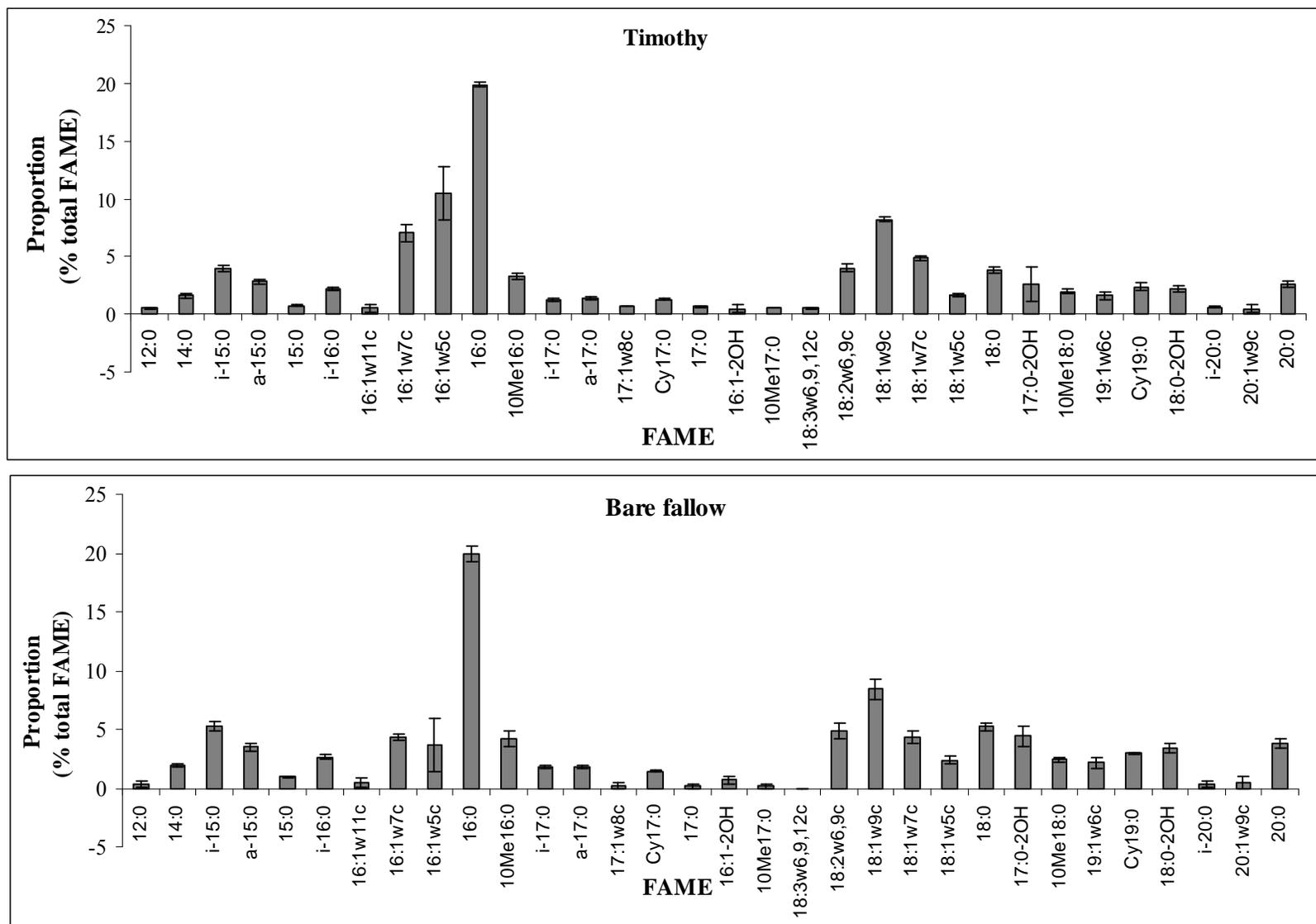
Figure A.3: Fatty acid methyl ester (FAME) profiles under the different cropping systems after three growing seasons. The vertical bars represent 2 x SE mean (n=4).

Table A.1: Effect of plant material addition on total soil fatty acid methyl ester (FAME) content (n=3).

Plant material	3 days	1 week	2 weeks	7 weeks	13 weeks	16 weeks	28 weeks
	Time since addition of plant material to soil						
	Total FAME (ug.g^{-1} dry soil)						
CL†	43,0 (6,2)[a]	57,7 (2,1)	74,3 (3,2)	152,7 (9,6)	145,7 (7,1)	73,7 (3,5)	76,3 (8,1)
CR	45,0 (2,0)	51,7 (3,2)	69,7 (3,8)	139,7 (2,1)	139,3 (4,5)	67,7 (2,1)	65,3 (1,2)
RCL	60,7 (2,9)	55,0 (7,9)	67,7 (2,1)	123,7 (1,5)	71,7 (3,1)	61,3 (3,2)	61,7 (0,6)
RCL	59,0 (2,0)	64,7 (2,1)	69,0 (1,0)	127,0 (3,0)	74,3 (3,1)	65,3 (5,0)	63,7 (4,2)
SL	52,7 (3,1)	60,3 (4,9)	77,7 (5,0)	103,7 (34,1)	87,7 (2,1)	72,7 (2,9)	83,0 (8,7)
SR	50,7 (5,7)	58,3 (5,5)	69,7 (3,1)	74,7 (3,5)	80,3 (3,8)	69,7 (5,1)	67,7 (3,8)
TL	59,0 (3,0)	71,7 (0,6)	73,3 (2,5)	144,7 (4,0)	78,3 (1,5)	66,0 (2,0)	66,0 (2,6)
TR	47,3 (1,5)	50,3 (3,2)	53,3 (4,0)	119,3 (7,1)	63,7 (7,6)	59,0 (2,0)	54,3 (4,0)
Soil	44,7 (1,5)	45,3 (4,7)	44,7 (6,4)	86,0 (1,0)	50,7 (3,1)	44,0 (2,0)	41,7 (2,3)

†CL, corn (leaves+stems); CR, corn roots; SL, soybean (leaves+stems); SR, soybean roots; RCL, red clover (leaves+stems); RCR, red clover roots; TL timothy (leaves+stems); TR, timothy roots; and Soil, no material addition. [a]Numbers in brackets are standard deviations.

Table A.2: Effect of plant material addition on CO_2 production (n=4).

	Time interval since amendment addition to soil (days)									
	0-3	3-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63
Plant material	CO_2 production (mg CO_2-C Kg^{-1} dry soil)									
CL†	138bc	115a	164a	82a	59a	38a	22a	23a	17a	12a
CR	104de	47de	65cde	62b	41b	35a	24a	21ab	18a	10a
RCL	147abc	64cd	75bcd	39cde	24cd	17b	17abc	17ab	14a	13a
RCR	160ab	66cd	78bcd	38cde	26cd	21b	15bc	18ab	15a	12a
SL	123cd	102ab	93bc	55bc	33bc	18b	17abc	16bc	14a	15a
SR	82ef	82bc	127ab	34de	20d	23b	17abc	15bc	16a	15a
TL	174a	56de	58cde	44cd	28cd	24b	19ab	18ab	19a	16a
TR	52f	34ef	42de	45bcd	33bc	24b	18abc	19ab	19a	15a
Soil	17g	14f	19e	24e	22cd	17b	12c	10c	12a	10a

	Time interval since amendment addition to soil (days)									
	63-70	70-77	77-84	84-91	91-98	105-112	112-119	119-126	126-133	133-140
Plant material	CO_2 production (mg CO_2-C Kg^{-1} dry soil)									
CL	14a	18ab	16abc	19a	12a	8b	13ab	12a	13bc	14abc
CR	14a	17bcd	20a	17ab	14a	10ab	10b	15a	18a	16ab
RCL	11a	17abcd	14bc	16ab	17a	11ab	16a	15a	13b	11c
RCR	12a	15d	15bc	13bc	15a	8b	14ab	12a	12bc	14abc
SL	14a	15cd	17abc	18ab	15a	13a	14ab	14a	12bc	17a
SR	12a	18ab	17ab	19a	16a	14a	15a	15a	13bc	14abc
TL	14a	20a	15abc	17ab	14a	10ab	12ab	13a	9bc	12abc
TR	13a	18abc	13c	16ab	17a	14a	12ab	13a	11bc	12bc
Soil	9a	15cd	13bc	9c	14a	9ab	11ab	14a	8c	10c

†CL, corn (leaves+stems); CR, corn roots; SL, soybean (leaves+stems); SR, soybean roots; RCL, red clover (leaves+stems); RCR, red clover roots; TL timothy (leaves+stems); TR, timothy roots; and Soil, no material addition. Within a column, numbers followed by the same letter(s) are not different at LSD 0.05 level.

Table A.2: Continued.

| Plant material | Time interval since amendment addition to soil (days) | | | | | | | | |
	140-147	147-154	154-161	161-168	168-175	175-182	182-189	189-196	0-196
	CO_2 production (mg CO_2-C Kg^{-1} dry soil)								
CL†	17abc	12abc	12ab	9c	14a	11a	9abc	9b	927a
CR	20a	13ab	17a	13ab	12ab	12a	14a	10b	708bc
RCL	14cd	12abc	10b	10bc	13ab	12a	11abc	11b	649cd
RCR	14bcd	14a	14ab	14ab	12ab	11a	11abc	11ab	660bcd
SL	7e	9c	15ab	14ab	12ab	15a	14a	10b	765b
SR	18ab	14ab	12ab	16a	12ab	13a	13ab	16a	665bcd
TL	11de	9bc	12ab	14ab	13ab	10a	9bc	11ab	724bc
TR	14bcd	11abc	13ab	15a	13ab	11a	7c	11ab	554d
Soil	12d	11abc	11b	14ab	9b	10a	12ab	8b	376e

†CL, corn (leaves+stems); CR, corn roots; SL, soybean (leaves+stems); SR, soybean roots; RCL, red clover (leaves+stems); RCR, red clover roots; TL timothy (leaves+stems); TR, timothy roots; and Soil, no material addition. Within a column, numbers followed by the same letter(s) are not different at LSD 0.05 level.