

**Conservation tillage in Kenya: the biophysical processes  
affecting its effectiveness**

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To my parents William and Gladys Maguta  
for the price they paid to  
take me to school

## ABSTRACT

Appropriate soil management is important for improved ecosystem functioning and high crop production. This study investigates how different tillage [reduced tillage (RT) and conventional tillage (CT)], crop residue (plus and minus crop residue) and cropping systems (soybean-maize intercropping, rotation and continuous maize) affected (i) soil aggregation, (ii) composition and diversity of microbial populations, (iii) crop residue (CR) disappearance and termite activity, (iv) nitrogen fixation and (v) crop productivity in Kenya. The main experiment in Nyabeda (western Kenya) had been established in 2003, while experiments in Matayos (western Kenya) and Machang'a (eastern Kenya) were established in 2005. Soybean-maize intercropping improved macroaggregation and reduced microaggregates and free silt and clay ( $P<0.05$ ) compared with the other cropping systems. The proportion of soil large macroaggregates was 30% to 89% higher in RT than in CT, depending on depth. Addition of CR affected ( $P<0.05$ ) soil aggregation mainly at the top 5 cm; it increased the large macroaggregates (by up to 180%) in the soybean-maize intercropping systems.

The composition of both bacteria and fungi communities was markedly different in the two tillage systems. With CR application, Simpson's indices of fungi were in the order intercropping > rotation > continuous maize. In addition, intercropping had highest bacteria diversity indices in the Nyabeda site. CR affected bacteria composition (e.g., in Matayos) and lowered diversity of soil fungi ( $P<0.01$ ); fungi Simpson's index was 0.75 for plots without and 0.65 for plots with crop residue. Bacteria diversity was inversely related to silt and clay. Fungi diversity (Simpson's index  $<0.7$ ) was highly inversely related with aggregate mean weight diameter and with soil hot water-extractable carbon.

CR disappearance was up to 85% of the initial residue in 3.5 months, and the relative contribution of macro- and mesofauna to residue disappearance was 70-95% for surface-placed and 30-70% for buried residues. Soil of termite galleries (mainly sheetings) was more enriched in carbon (1.6%) than bulk farm soil (1.4%) and mound soil (1.2%;  $P<0.01$ ); gallery soil and bulk farm soil had similar aggregates sizes but the values were lower (22-56% for  $>250<2000$   $\mu\text{m}$  aggregates;  $P<0.05$ ) than for mound soil.

Soybean nitrogen derived from the atmosphere (%NDfA) ranged from 42-65%; it was higher ( $P<0.05$ ) in RT (55.6%) than in CT (48.2%). Nitrogen fixed seasonally in soybean aboveground plant parts was 26-48 kg N ha<sup>-1</sup> with intercropping and 53-82 kg N ha<sup>-1</sup> with rotation. Seasonal litter-fall contained about 15 kg N ha<sup>-1</sup>. Total fixed N under RT plus CR was at least 55% and 34% higher than in the other treatments (RT minus CR, CT plus CR, and CT minus CR) in intercropping and rotation systems, respectively.

Seasonal average maize grain yields were 3.2-4.1 t ha<sup>-1</sup> in continuous maize, 3.0-3.9 t ha<sup>-1</sup> in soybean-maize rotation, and 1.8-2.8 t ha<sup>-1</sup> in the soybean-maize intercropping system. Soybean grain yields were 0.92-0.99 t ha<sup>-1</sup> in the soybean-maize rotation and 0.52-0.60 t ha<sup>-1</sup> in the intercropping system. The net benefits were highest in the soybean-maize intercropping, followed by rotation > continuous maize. Soybean yields were similar between CT and RT; maize yields were lower ( $P<0.05$ ) in RT than CT. Overall net benefits for the 9 seasons were higher in CT than in RT.

We conclude that (i) despite fast disappearance of CR, its application increases soil aggregation and influences microbial composition and diversity and nitrogen fixation; (ii) for Ferralsols of western Kenya, combining RT and CR is important for improved soil structural stability and, intercropping maize and legume (soybean) leads to better soil structure and also gives higher net benefits than conventional rotation and continuous maize systems; and (iii) RT is appropriate for soybean production; maize yields are lower in RT than in CT due to surface crusting in the RT resulting from inadequate soil cover.

## KURZFASSUNG

### **Ressourcenschonende Landwirtschaft in Kenia: Die ihre Effektivität beeinflussenden biophysikalischen Prozesse**

Eine richtige Bodenbearbeitung ist wichtig für die verbesserte Funktion von Ökosystemen und für hohe landwirtschaftliche Erträge. Diese Studie untersucht den Einfluss verschiedener Bodenbearbeitungsmethoden [reduzierte Bodenbearbeitung (*reduced tillage*; RT) und konventionelle Bodenbearbeitung (*conventional tillage*; CT)], Ernterückstände (mit und ohne Rückstände) und Anbausysteme (Sojabohnen-Mais Mischkultur, Rotation und fortlaufender Maisanbau) auf (i) Bodenaggregation, (ii) Zusammensetzung und Diversität von Bodenmikrobengemeinschaften, (iii) Verschwinden von Ernterückständen (*crop residue*; CR) und Aktivität von Termiten, (iv) Stickstofffixierung (N) und (v) landwirtschaftliche Produktivität in Kenia. Die Hauptuntersuchungsfläche in Nyabeda (Westkenia) bestand seit 2003, während die Untersuchungen in Matayos (Westkenia) und Machang'a (Ostkenia) in 2005 begonnen wurden. Mit Sojabohnen-Mais-Zwischenpflanzung verbesserte sich die Makrostruktur des Bodens, während die Mikrostruktur und freier Schluff bzw. Ton ( $P < 0.05$ ) reduziert waren im Vergleich zu den anderen Anbausystemen. Abhängig von der Bodentiefe war der Anteil der groben Bodenstruktur 30% bis 89% höher bei RT als bei CT. Der Zusatz von Pflanzenrückständen beeinflusste ( $P < 0.05$ ) die Bodenstruktur hauptsächlich in den oberen 5 cm; der Anteil der groben Bodenstruktur nahm bis zu 180% bei Sojabohnen-Mais-Zwischenpflanzung zu.

Die Zusammensetzung sowohl der Bakterien- als auch der Pilzgemeinschaften unterschied sich deutlich in den beiden anderen Systemen. Die Simpson-Indices der Pilze sanken mit Anwendung von Pflanzenrückständen in der Folge Zwischenpflanzung >Rotation >ununterbrochener Maisanbau, und Zwischenpflanzung zeigte die höchsten Bakteriendiversitätsindices am Standort in Nyabeda. Pflanzenrückstände beeinflussten die Bakterienzusammensetzung (z.B. in Matayos) und reduzierten die Diversität von Bodenpilzen ( $P < 0.01$ ); der Simpson-Index war 0.75 für Flächen ohne bzw. 0.65 für Flächen mit Rückständen. Bakterielle Diversität war umgekehrt proportional zu Schluff und Ton. Pilzdiversität (Simpson- index  $< 0.7$ ) war stark umgekehrt proportional zum Durchmesser des mittleren Gewichts der Bodenstrukturanteile und zum mit heißem Wasser extrahiertem Kohlenstoff.

Bis zu 85% der ursprünglichen Pflanzenrückstände verschwand in 3.5 Monaten und der relative Beitrag der Makro-bzw. Mesofauna hierzu war 70-95% für oberflächlich

ausgebrachte bzw. 30-70% für eingearbeitete Rückstände. Der Boden der Termitengalerien (hauptsächlich überbaute Laufwege) enthielt mehr Kohlenstoff (1.6%) als Farmboden (1.4%) und Bodenmaterial in Termitenhügeln (1.2%;  $P < 0.01$ ); die Größe der Aggregate in Farmboden und Galerien war ähnlich, aber niedriger (22-56% für  $>250 < 2000 \mu\text{m}$  Aggregate;  $P < 0.05$ ) als die des Bodenmaterials in Termitenhügeln.

Sojabohnenstickstoff aus der Atmosphäre (%NDfA) war höher ( $P < 0.05$ ) bei RT (55.6%) als bei CT (48.2%). Jahreszeitlich abhängige Streu enthielt ca.  $15 \text{ kg N ha}^{-1}$ . Gesamtfixierter Stickstoff bei RT plus CR war mindestens 55% bzw. 34% höher als bei den anderen Bodenbehandlungen (RT minus CR, CT plus CR, bzw. CT minus CR) in den Zwischenpflanzungs- bzw. Rotationssystemen.

Die jahreszeitlich abhängigen durchschnittlichen Sojabohnenerträge waren ähnlich bei CT und RT; Maiserträge waren niedriger ( $P < 0.05$ ) bei RT als bei CT. Der gesamte Nettonutzen für die 9 Jahreszeiten war höher bei CT als bei RT. Die Nettonutzen waren am höchsten bei der Sojabohnen-Mais-Zwischenpflanzung gefolgt von Rotation > ununterbrochener Maisanbau.

Es kann daher davon ausgegangen werden, dass (i) trotz des vollständigen Verschwindens, Pflanzenrückstände die Bodenaggregation erhöhen und die Zusammensetzung und Diversität der Bodenmikroben sowie die Stickstofffixierung beeinflussen; (ii) für die Ferralsols von Westkenia die Kombination von RT und Pflanzenrückständen wichtig ist für eine verbesserte strukturelle Stabilität der Böden, während Zwischenpflanzung von Mais und Hülsenfrüchten (Sojabohnen) zu einer verbesserten Bodenstruktur und auch zu höheren Nettonutzen im Vergleich zur konventionellen Rotation bzw. zu ununterbrochenem Maisanbau führen, und (iii) RT richtig ist für die Sojabohnenproduktion; Maiserträge sind niedriger bei RT als bei CT durch die Oberflächenverkrustung bei RT wegen der unzureichenden Bodenbedeckung.

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## LIST OF ABBREVIATIONS AND ACRONYMS

CIAT	International Center for Tropical Agriculture (Centro Internacional de Agricultura Tropical).
CIMMYT	International Maize and Wheat Improvement Center (Centro Internacional de Mejoramiento de Maizy Trigo)
DNA	Deoxyribonucleic acid
FAO	Food and Agricultural Organization of the United Nations
IAEA	International Atomic Energy Agency
ICRAF	International Center for Research in Agroforestry (currently the World Agroforestry Center)
PCA	Principal component analysis
PCR-DGGE	Polymerase chain reaction - denaturing gradient gel electrophoresis
Reduced tillage	In the context of this thesis, it is a conservation agriculture practice where minimum soil disturbance, the first principle of conservation agriculture, is achieved through tillage restricted to the top 3 cm of the soil and only in the areas with weeds. The practice is employed in various combinations with the other key principles of conservation agriculture, i.e., keeping the soil surface covered by crop residue, and adopting crop rotation/sequencing over time and space.
SOM	Soil organic matter

## **1 GENERAL INTRODUCTION**

### **1.1 Background**

Intensive soil tillage generally leads to soil degradation and loss of productivity (Derpsch 2008) and has led to calls for introducing conservation agriculture (CA) practices with minimum soil disturbance (McCarty and Meisinger 1997; Wander and Yang 2000). Advantages of CA include erosion control, increased infiltration, soil moisture conservation, reduced costs of fuel consumption (Benites 2008; Derpsch 2008) and drudgery (Landers 2008), improved soil ecology and nutrient cycling (Blank 2008), food security, increased incomes and a variety of environmental services (reduced CO<sub>2</sub> emissions, less flood damage, cleaner surface waters, (Landers 2008)). Important principles of CA have been identified as minimal soil disturbance, surface residue retention and crop rotation (Erenstein et al. 2008) and additionally, restricted in-field traffic (Benites 2008). The CA concept encompasses practices such as mulch-based no-till systems (known variably as zero-tillage or direct seeding) with soil disturbance restricted to seed sowing (Erenstein et al. 2008); and conservation tillage involving some form of soil disturbance, e.g., strip-tillage, ripping and subsoiling, ridging, and varied locally-adapted reduced tillage practices (Erenstein et al. 2008). In Kenya, conservation tillage practices involve use of mulch, ripping and sub-soiling without inverting soil (Gitonga et al. 2008). Although practiced by large-scale farmers especially in the Mount Kenya region, conservation tillage is slowly being adopted by some small-scale farmers, and evaluating its performance in these conditions is presently a priority. Use of herbicides for weed control is not a common practice in Kenya, and means of mechanical weeding is one of the issues being investigated (Gitonga et al. 2008). The Tropical Soil Biology and Fertility institute of CIAT (TSBF/CIAT) initiated a form of conservation tillage involving the use of hand-hoes and weeding restricted to scratching the top 0-3 cm soil, only in the parts with weeds. This is referred to here as reduced tillage (RT).

At the level of small-scale farmers, one of the major challenges to the practice of reduced tillage is the availability of sufficient crop residues for mulch. This is a common problem in many regions (Erenstein 2003; Tursunov 2009), and also in sub-Saharan Africa (Bationo et al. 2007; Fowler and Rockstrom 2001). Recommendations

suggest that at least 30% of soil surface cover with crop residue would be required at planting in order to have the expected effects in CA (Fowler and Rockstrom 2001). But the effect of the mulch in the conservation practice varies according to the mulch type and quantity (Scopel et al. 1998) as well as to the rate of disappearance of the residue through processes such as comminution by termites. The source of mulch is often crop residue from the previous season, mainly cereal. Legumes in association with cereal could make a significant contribution to the quantity and quality of residue mulch in conservation tillage systems. Including legumes in the cropping system has been shown to have both short-term benefits (increased water and nutrient use efficiency, yields and economic returns) and long-term effects (increased N-supplying power of the soil, microbial diversity and carbon sequestration (Gan and Goddard 2008)).

The importance of soil micro-organisms, specifically fungi and bacteria, in soil nutrient cycling and soil structure is underscored by many researchers (Backmann et al. 2003; Bremer et al. 2007). Unfortunately, the composition of soil micro-organism assemblages is affected by management practices, and compositional shifts can result due to different tillage and crop residue regimes. Rapid molecular tools, such as polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), are now available to characterize soil microbial communities and their changes due to different management practices. Within eastern Africa, the extent of tillage and crop residue on microbial compositional shifts is not yet well studied, and there is no study that compares microbial communities in the cropping systems used commonly by farmers in the region.

Reduced tillage practices can enhance soil fauna and microbial activities that are vital in formation and stabilization of aggregates, thus improving soil structure (Craswell and Lefroy 2001; Feller et al. 2001). The aggregates themselves reinforce and protect soil organic matter (SOM) from erosion and mineralization losses (Feller et al. 2001). The stability of aggregates resulting from processing by soil biota depends on the organic matter egested (Lavelle et al. 2001). Both soil aggregation and soil biota activity, vital for nutrient turnover and ultimately soil fertility and crop productivity, are affected by management practices (Martius et al. 2001). Developing crop management technologies that increase soil fertility and soil organic carbon (SOC) has been identified as a major challenge at the biophysical level (Craswell and Lefroy 2001).

Martius et al. (2001) opined that the role of soil biota is rarely considered or understood in agricultural studies, while Machado and Silva (2001) suggested a need to further investigate the influence of reduced tillage and legume-cereal association on aggregate stability. Soil aggregation has not been well studied under African Ferralsols and tropical sub-humid conditions. Indeed, for such Ferralsols where inorganic (iron and aluminium oxides) bonding agents and dominating 1:1 kaolinite clay mineral also contribute to soil aggregation, the aggregate hierarchy concept (where aggregates form around organic matter) is still in dispute (Lima et al. 2000). Also, no work has compared soil aggregation concurrently under the three main cropping systems used by farmers (legume/cereal intercropping, legume-cereal rotation and continuous cereal).

The unique socio-economic conditions in sub-Saharan Africa limit access to mineral fertilizers, inducing the search for cheap sources of fertilization, such as nitrogen fixation in grain legumes. Soybean production in Kenya has been increasing in recent years. Its production offers the opportunity for small-scale farmers to benefit from biological nitrogen fixation and to reduce soil N mining, which is estimated at 22 kg N ha<sup>-1</sup> for sub-Saharan Africa (Stoorvogel et al. 1993). Biological nitrogen fixation under different tillage practices is, however, not yet well studied in Africa, and few studies have been conducted in eastern Africa.

### **1.2 Research justification**

Soil degradation is a common problem resulting in low crop yields in many parts of eastern Africa, as also in other parts of sub-Saharan Africa. Reduced tillage offers an opportunity to reduce soil degradation and increase both benefits to the farmer and the society through provision of private and public goods and services. In Africa, crops are mainly produced in small-scale land holdings, and the benefits of reduced tillage are likely to be greatest in these. This study was therefore conducted under smallholder farm conditions. To make appropriate recommendations from a better understanding of the effects of different technologies, a long-term assessment of reduced tillage is necessary. This study is based on an assessment of up to ten seasons of experimentation. Soil structure, microbial diversity and biological nitrogen fixation studied after such a long period of experimentation provide better insight into the processes characterizing

the different tillage systems. Such understanding provides an opportunity to better manage crop production systems and increase yields.

### **1.3 Objectives and hypotheses**

This study aimed to understand the biophysical processes affecting effectiveness of reduced tillage in Kenya, and to recommend best management practices for smallholder farmers. The main goal of this study was to find out how tillage system, crop residue and cropping systems affect soil and crop productivity. Specific objectives were:

1. To determine the effects of tillage, crop residue application and cropping system on soil aggregation in western Kenya,
2. To characterize microbial diversity and composition in different tillage, crop residue and cropping systems in western and eastern Kenya using PCR-DGGE techniques,
3. To assess the rate of crop residue disappearance and determine the characteristics (distribution, aggregation and nutrient contents) of biogenic (termitogenic) structures in cropping systems in western Kenya,
4. To determine the effect of tillage and crop residue on legume nitrogen ( $N_2$ ) fixation in maize-soybean intercropping and rotation systems in western Kenya, using the  $^{15}N$  isotope dilution approach, and,
5. To determine crop productivity in different tillage practices, crop residue management and cropping systems over several seasons in western Kenya.

The overarching hypothesis was that reduced tillage combined with surface crop residue application and mixed cropping, involving a combination of crop species, is superior in improvement of soil quality, and that no differences in yield would be observed between it and a conventional tillage system.

The following hypotheses were tested:

1. Despite the dominating nature of oxidic bonding agents in Ferralsols, reduced tillage systems with less disturbance have higher portions of macroaggregates

- and of microaggregates protected within macroaggregates compared with systems under periodic disturbance, and cropping systems involving legumes increase soil aggregation relative to continuous cereal monocropping systems,
2. The diversity of soil micro-organism assemblages is higher under 'reduced tillage plus crop residue' and in legume-cereal cropping systems than under conventional tillage systems and continuous cereal cropping,
  3. Soil macro- and mesofauna contribute more to fast disappearance of applied crop residue in sub-humid western Kenya than micro-organisms,
  4. Combining reduced tillage and crop residue results in increased rates of biological nitrogen fixation when compared to conventional tillage systems, and
  5. With crop residue application in reduced tillage under the sub-humid conditions of western Kenya, similar crop yields to those in conventional tillage systems are obtained.

### **1.4 Study sites**

The study was conducted in Nyabeda (located in a sub-humid site in western Kenya), Matayos (also in sub-humid western Kenya) and Machang'a, (in a semi-arid zone in eastern Kenya; Figure 1.1). The zones are characterized by two rainy seasons: a long rainy season between March and August and short rainy season between September and February for the sub-humid zones, and long rainy season between May and August and short rainy season between October and January for the semi-arid zone. Maize is the main staple crop and is normally grown either as a monocrop or in association with legumes, mainly common beans and groundnuts. Soybean, also treated as a cash crop, is being adopted, especially in western Kenya. Both zones have predominantly smallholder settlements, with land sizes ranging from 0.3 to 3 ha per household. The sites represent different soil types with Nyabeda having clay, Matayos mixture of sand and clay and Machang'a within a sandy soil (Table 1.1).



### **1.5 General experimental design**

The study was executed within already established researcher-managed experiments being conducted by the African Network for Soil Biology and Fertility (AfNet) of the Tropical Soil Biology and Fertility (TSBF) Institute of CIAT. The two sites in western Kenya (Matayos and Nyabeda) were located on farmer fields, while the site in eastern Kenya (Machang'a) was located within a field under the Kenyan Ministry of Agriculture. The Nyabeda experiment was established in March 2003, while Matayos and Machang'a were both established in September 2005. Due to the longer period under experimentation in Nyabeda, most of the work was conducted in the Nyabeda experimental site.

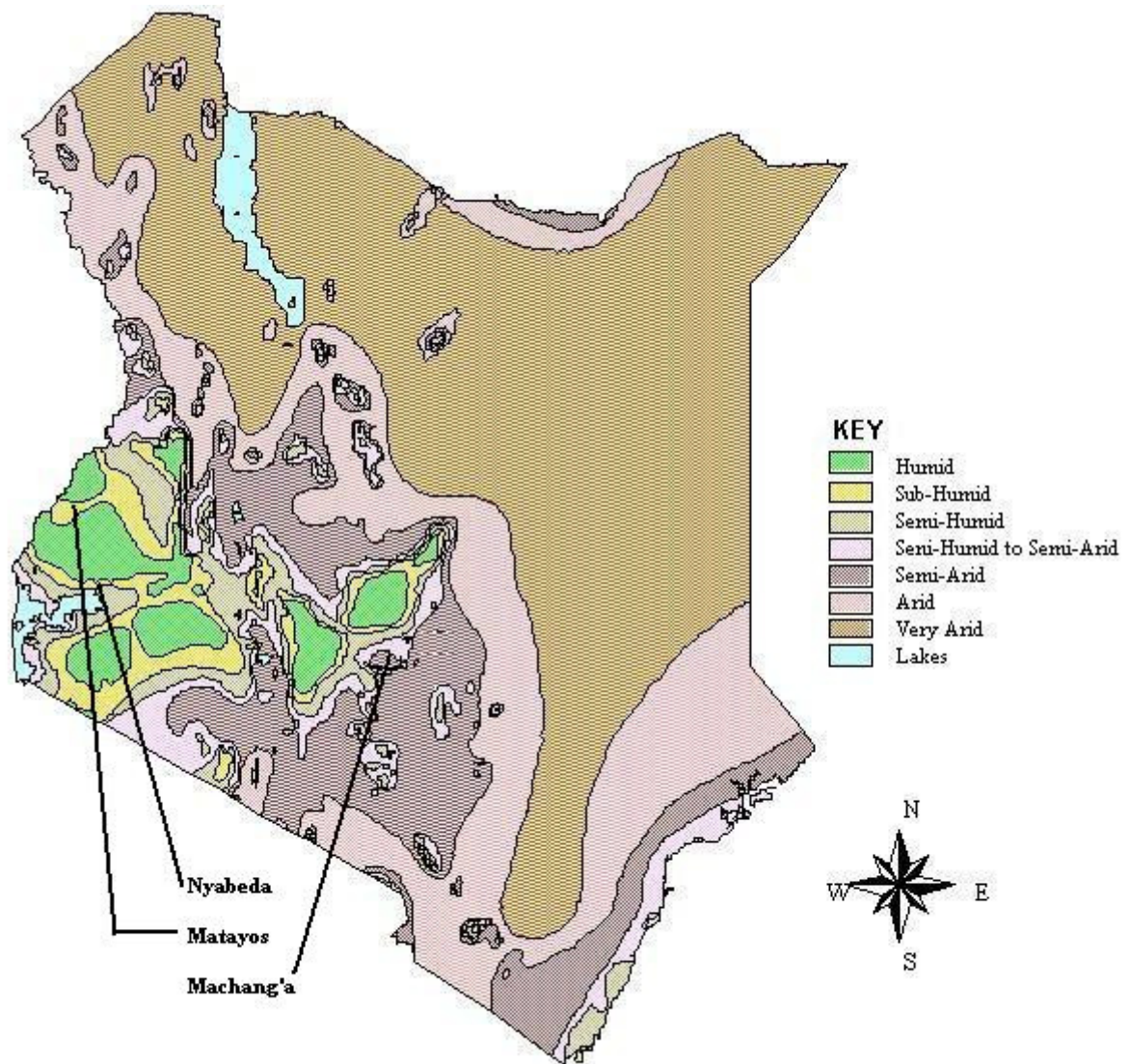


Figure 1.1. Location of conservation tillage experiments studied in Kenya. Source of map: United States Department of Agriculture (2004)

Table 1.1. Location, climatic and soil characteristics of the study sites

<i>Parameter</i>	<i>Machang'a</i>	<i>Matayos</i>	<i>Nyabeda</i>
Agro-climatic zone	Semi-arid	Sub-humid	Sub-humid
Agro-ecological zone	Lower midland 3 <sup>f</sup>	Lower midland 2 <sup>e</sup>	Lower midland 2
Latitude	0° 45' S <sup>a</sup>	0° 22' N	0° 07' N
Longitude	37° 45' E <sup>a</sup>	34° 10' E	34° 24' E
Altitude (m.a.s.l.)	1050	1200	1420
Total annual rainfall (mm)	560-1250 <sup>c</sup>	1400-1700 <sup>e</sup>	1200-1600 <sup>d</sup>
Daily temperatures (°C):			
Mean	23.4 (±1.53)	22 <sup>e</sup>	23.2 (±1.52)
Minimum	17	16 <sup>e</sup>	14
Maximum	30	28 <sup>e</sup>	31
Soil type	Chromic Cambisols <sup>a</sup>	Orthic Acrisols <sup>e</sup>	Ferralsol <sup>b</sup>
Sand:silt:clay ratio	65:13:22	37:25:38	15:21:64
pH (water)	6.46 (±0.46)	5.6 (±0.26)	5.08 (±0.27)
Extractable K (me 100g <sup>-1</sup> )	0.56 (±0.14)	0.26 (±0.08)	0.10 (±0.04)
P (mg P kg <sup>-1</sup> )	6.3 (±7.74)	47.5 (±20.2)	2.99 (±2.09)
Ca (cmolc kg <sup>-1</sup> )	2.29 (±1.04)*	3.92 (±0.41)	4.69 (±0.33)*
Mg (cmolc kg <sup>-1</sup> )	0.78 (±0.08)*	1.15 (±0.13)	1.68 (±0.13)*
Total SOC (%)	0.61 (±0.07)	1.17 (±0.13)	1.35 (±0.06)
Total nitrogen (%)	0.05 (±0.01)	0.12 (±0.01)	0.15 (±0.02)

\*value obtained in meq 100g<sup>-1</sup> of soil; <sup>a</sup> Warren et al. (1997); <sup>b</sup> Sombroek et al. (1980); <sup>c</sup> 2002-2007 period; <sup>d</sup> 2003-2008 period; <sup>e</sup>see Jaetzold et al. (2007) for details; <sup>f</sup>see Jaetzold and Schmidt (1983) for details

### **1.5.1 Experimental design and crop management in Nyabeda**

The experiment was set up as a split-split-split plot involving a factorial combination of tillage systems in the main plots [reduced tillage (RT) and conventional tillage (CT)], crop residue management in split plots (plus and minus crop residue), cropping system in split-split plots (continuous cereal, rotation, intercropping; Table 1.2) and nitrogen (N) or phosphorus (P) in split-split-split plots, and was replicated four times (Figure 1.2). Subplots in the continuous cereal system had four levels of N fertilizer (0, 30, 60 and 90 kg N ha<sup>-1</sup>) applied to assess N fertilizer response and equivalence of the rotation system. For the rotation, two subplots (one planted with maize and the other with a legume) had no N, while the other two received fertilizer N at 60 kg N ha<sup>-1</sup>. In addition, two extra subplots were appended in the rotation system that did not receive either fertilizer N or P. Subplots under intercropping were not applied with N. Except for the two appended subplots under the legume-maize rotation system, all plots in this site were applied with fertilizer P (triple super phosphate) at 60 kg ha<sup>-1</sup>. Subplots at fertilizer level were 4.5 m by 7 m, with 1 m left between the plots. The N fertilizer was in the form of urea, split-applied with one-third at planting and two-thirds at plant knee height (four weeks after planting). All the plots were applied with potassium (K) at 60 kg K ha<sup>-1</sup>. The application method of fertilizers was hill placement. The experiment was surrounded by a number of border rows to which N and P fertilizer was applied. Prior to establishment of the experiment, the farm had been under a native vegetation of grasses and shrubs.

Table 1.2. Treatments combining tillage practice, crop residue and cropping system in Nyabeda, western Kenya

Treat. no.	Tillage method	Crop residue	Cropping system
1	Reduced tillage	-Crop residue	Legume-cereal rotation
2	„	„	Legume-cereal intercropping
3	„	„	Continuous cereal
4	„	+Crop residue	Legume-cereal rotation
5	„	„	Legume-cereal intercropping
6	„	„	Continuous cereal
7	Conventional tillage	-Crop residue	Legume-cereal rotation
8	„	„	Legume-cereal intercropping
9	„	„	Continuous cereal
10	„	+Crop residue	Legume-cereal rotation
11	„	„	Legume-cereal intercropping
12	„	„	Continuous cereal

Crop residue (maize stover) was applied at 2 t ha<sup>-1</sup>

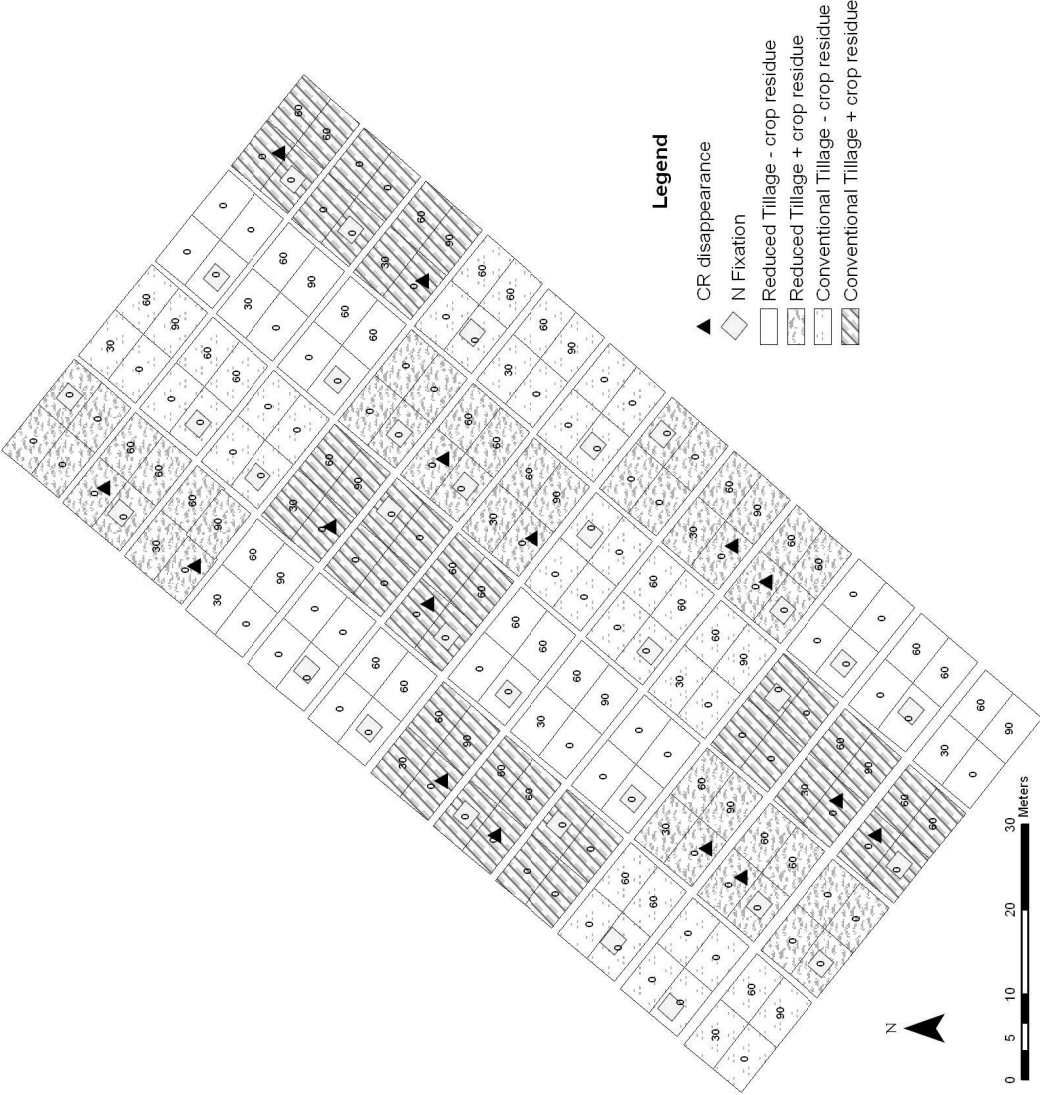


Figure 1.2. Layout of the conservation tillage experiment in Nyabeda showing tillage and crop residue combination treatments and plots used for assessment of nitrogen fixation and crop residue disappearance; values inside plots represent, nitrogen applied in  $\text{kg N ha}^{-1}$

At the start of the experiment, land preparation was done uniformly across all plots by hand-ploughing to 15 cm depth. Subsequent tilling operations were hand-hoeing up to about 10 cm depth in conventional tillage, and surface scratching to about 3 cm depth only in portions with weeds in reduced tillage. There were two weeding operations and one handpulling operation under continuous cereal and intercropping for each of the seasons. For rotation, while the part under maize was weeded in the same way as the continuous cereal plots, the part under soybean was weeded once and handpulled once every season. Crop residue (maize stover) was applied seasonally at 2 t ha<sup>-1</sup> before planting, while fertilizers were hill-placed at the time of sowing. Maize (*Zea mays* L.) varieties were Hybrid 513 in season 1, Hybrid 502 (from Western Seed company) in season 2, 3, 6 and 8, Imidazolinone-Resistant (IR) maize in season 4, 7 and 9, and hybrid 403 (also from Western Seed company) in season 5. For the legumes, common bean (*Phaseolus vulgaris* L.) was grown in season one and soybean (*Glycine max* (L.) Merr; TGX 1448-2E) was grown in the subsequent seasons (planting and harvest dates in Table 1.3). Legume residues were left on the respective plots. Rainfall at the Nyabeda experimental site was measured daily using a simple rain gauge installed in the experimental farm (Table 1.3).

Table 1.3. Cropping calendar and rainfall during the experimental period in Nyabeda, western Kenya

Season	Planting date	Harvest date	Seasonal rainfall (mm)	Number of rainy days
1	1 April 2003	24 August 2003	697	56
2	6 September 2003	15 January 2004	402	40
3	17 March 2004	3 August 2004	593	50
4	30 August 2004	12 January 2005	690	53
5	1 April 2005	12 August 2005	625	54
6	27 September 2005	24 February 2006	406	38
7	28 March 2006	7 August 2006	986	62
8	5 September 2006	26 January 2007	935	74
9	4 April 2007	27 August 2007	712	50

### 1.5.2 Experimental design in Matayos

The experiment in Matayos was done following the same design and management as the one in Nyabeda, except that it started two years later, in 2005.

### 1.5.3 Experimental design in Machang'a

The experiment in the semi-arid site Machang'a was a randomized complete block design with three tillage systems (conventional tillage, no-till and tied-ridging) each with two organic applications (2 t ha<sup>-1</sup> manure applied alone and, 1 t ha<sup>-1</sup> manure plus 1 t ha<sup>-1</sup> crop residue; Table 1.4). The cropping system was continuous monocropping of *Zea mays* (Katumani composite variety that takes 72 days to mature). No-tillage involved hand-pulling as the weed management system. Tied-ridges were implemented as reduced tillage, with tillage restricted to maintenance of the ridges. Conventional tillage was similar to that of the other sites.

Table 1.4. Tillage and organic resource treatments implemented in Machang'a, eastern Kenya

<i>Treat no</i>	<i>Tillage system</i>	<i>Organic resource</i>
1	Conventional tillage	- Residue (absolute control)
2	Conventional tillage	+ Crop residue (1 t ha <sup>-1</sup> ) + manure (1 t ha <sup>-1</sup> )
3	Conventional tillage	+ Manure only (2 t ha <sup>-1</sup> )
4	No-tillage	+ Crop residue (1 t ha <sup>-1</sup> ) + manure (1 t ha <sup>-1</sup> )
5	No-tillage	+ Manure only (2 t ha <sup>-1</sup> )
6	Tied-ridges	+ Crop residue (1 t ha <sup>-1</sup> ) + manure (1 t ha <sup>-1</sup> )
7	Tied-ridges	+ Manure only (2 t ha <sup>-1</sup> )

### 1.6 Soil and plant analyses

Soil samples taken at experiment initiation from 0-15 cm depth were bulked per replicate and analyzed for total C and N, available P and K, exchange Ca and Mg, texture and pH. Additionally, soil and plant analyses were done on samples taken during



the ninth and tenth seasons. Soil total N was based on wet oxidation using Kjeldahl digestion, while soil total C was extracted using acidified dichromate (Anderson and Ingram 1993). Soil pH was determined in water, while exchangeable Ca and Mg were extracted using 1 N KCl extractant and determined using a spectrophotometer. Analyses for extractable inorganic phosphorus and exchangeable potassium were based on a modified Olsen extractant, 0.5 M NaHCO<sub>3</sub> + 0.01 M EDTA, pH 8.5, (ICRAF 1995; Okalebo et al. 2002). Analyses for plant N, P and K was by wet oxidation based on Kjeldahl digestion with sulphuric acid (Parkinson and Allen 1975). Soil texture was determined using a Bouyoucous hydrometer (Hydrometer method) (Gee and Bauder 1986). These analyses were done at the ICRAF and TSBF laboratories in Nairobi.

### **1.7 Thesis structure**

This thesis has a total of seven chapters. Chapter 2 analyses soil in the context of aggregate size distribution. Chapter 3 deals with bacteria and fungi composition and diversity in conservation tillage systems in sub-humid and arid zones in Kenya. Chapter 4 discusses crop residue disappearance and termite activity over the growing season, and in Chapter 5, the effects of tillage and crop residue on soybean nitrogen fixation, a functioning of soil micro-organisms, are discussed. Chapter 6 discusses the crop yield data obtained over several seasons. In the final Chapter 7, the general discussion, summary and recommendations are presented.

## **2 SOIL AGGREGATION AND AGGREGATION INDICES AS AFFECTED BY TILLAGE, CROPPING SYSTEMS AND CROP RESIDUE APPLICATION**

### **2.1 Introduction**

The arrangement of soil particles into different aggregates determines soil structure and affects plant-soil nutrient and water relations. Frequently, soil aggregation occurs when soil particles adhere on organic debris (Six et al. 2000), and in temperate climates, the composition of aggregates usually follows the concept of aggregate hierarchy, i.e., larger aggregate units are composed of smaller ones with different types of organic bonding materials (Oades and Waters 1991; Tisdall and Oades 1982). The model of Six et al. (2000a), for instance, explains the transformation of coarse intra-aggregate particulate organic matter (iPOM) within macroaggregates to fine iPOM and encrustation of these fine iPOM with clay particles to form microaggregates. For oxidic soils such as those of the tropics, the aggregate hierarchy concept is still in dispute, since Fe and Al oxides largely determine pseudosilt and thus microaggregate stability (Lima et al. 2000). Also, Lima and Anderson (Lima and Anderson 1997) found no increase in carbon by aggregate size in a Brazilian oxisol, an indicator that oxides dominated aggregation. Hence, especially for such tropical climates, the role of soil organic matter (SOM) management for sustaining soil structure still warrants attention.

For Mollisols of temperate climates, it has been hypothesized that the quantity and quality of residues added or retained in the system have important effects on soil aggregation (Mrabet et al. 2001). The effects on aggregation depend on availability of the residue, on climate, which affects residue turnover (Andren et al. 2007; Angers 1998; Six et al. 2000a), and on the spatial-temporal arrangement of the crop components themselves. Intercropping has a continued supply of residues of different quality both on the soil surface as litterfall and belowground through sequential root dieback as opposed to rotation and continuous cropping where pure stands are implemented. With a legume present, there is likely a higher contribution of labile organic compounds and hence more transient and temporally binding agents (Degens 1997). In addition, crop combinations influence microbial diversity and density and therefore microbial exudates produced during POM decomposition. As such, legumes and cereals affect aggregation

differently depending on their arrangement on the farm. Although spatial-temporal arrangements of crop components in tropical east Africa vary from pure stands, continuously monocropped, to rotations and intercropping of cereals, legumes and tubers, no comparative study on soil aggregation within these management regimes under tropical climates was found. Indeed, there is very scanty information on soil aggregation for one of the most predominant African cropping systems, i.e., intercropping.

Crop residue after harvest is either left on the farm as a strategy for soil fertility management or removed to serve varied uses, e.g., fodder, fuel etc. The residue is usually maize stover, which is of lower quality than legume residues, and their interaction could lead to aggregation differences between systems where legume is included and those planted with cereal only. Besides crop residue, tillage is known to affect soil aggregation through disruption of macroaggregates and increased turnover rate of organic resources (Oades 1984; Zotarelli et al. 2007), and reducing tillage can promote growth of soil micro-organisms such as fungi that play a part in aggregating soils (Beare et al. 1993; Degens et al. 1996). The study investigated how maize stover retained within the cropping systems affects soil aggregation under conventional and reduced tillage systems.

The hypotheses were that

- Similar to other climates, also in the sub-humid tropics the reduced tillage system, with its lower disturbance, has higher portions of macroaggregates and of microaggregates protected within the macroaggregates compared with systems under periodic disturbance, despite the dominating nature of oxidic bonding agents in the Ferralsols, and
- Cropping systems involving legumes increase soil aggregation relative to continuous cereal monocropping systems.

These two hypotheses were assessed using wet-sieving and isolation of aggregates using soil samples from an existing split-split-plot on-farm, researcher-managed experiment in sub-humid Kenya.

## 2.2 Materials and methods

The study was conducted in 2007 in Nyabeda in western Kenya, in an experiment established as a long-term conservation tillage trial in March 2003 (for general soil and climatic characteristics see Table 1.1). The soil is a Ferralsol (Sombroek et al. 1980) and in terms of mineralogy, it contains 40% kaolinite, 18% quartz, 17% goethite, 3% potash feldspar, 2% hematite and anatase and 1% crandallite, while 18% is made up of amorphous compounds (Knut Ehlers, unpublished data).

The experiment was set up as a factorial design involving tillage (reduced and conventional tillage), crop residue (CR) application (maize stover applied at 0 and 2 t ha<sup>-1</sup>) and cropping systems (continuous cereal, legume/cereal intercropping and legume-cereal rotation). Under 'reduced tillage', tillage was restricted to surface scratching down to 3 cm depth to remove weeds, while conventional tillage involved tillage using hand-hoes to about 10-15 cm depth as done by small-scale farmers. Soybean (*Glycine max* (L.) Merr) (TGX 1448-2E, locally known as SB20) was used as the legume, while maize (*Zea mays*) was the cereal crop. Legume residues were left on the plots after harvest.

Soil sampling was done at four subsequent depths (0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm) using rings of 5 cm diameter and 5 cm height. Two replicates were sampled 14 weeks after planting in July 2007 (during the legume phase of the rotation), while the third replicate was sampled during the subsequent season in November 2007 at 7 weeks after planting. The samples were air-dried for four days to constant weight. Wet-sieving was done for each depth separately. First, the soil was passed through an 8 mm sieve from where 80 g was weighed for wet-sieving, and an additional 10 g for moisture content determination. The 80 g sample was submerged in water over a 2 mm sieve for 5 min to allow slaking, followed by sieving for 2 min to obtain large macroaggregates. Soil that passed through the sieve was sieved again for 2 min using a 250 µm sieve to obtain the small macroaggregates. The aggregates not captured by the 250 µm sieve were then sieved for 2 min using a 53 µm sieve to obtain the microaggregates. After sieving with a 53 µm sieve, 250 mm of the filtrate containing silt and clay was obtained. The four different samples were dried at 60°C for 48 to 72 hours before weighing. Sand content (used for sand correction) of the aggregates (>53 µm) was determined by dispersing a subsample of aggregates from selected treatments

with sodium hexametaphosphate. Mean weight diameter (MWD) of the aggregate fractions was calculated as

$$MWD = \sum X_i W_i \quad (2.1)$$

where  $X_i$  is the diameter of the  $i$ th sieve size and  $W_i$  is the proportion of the total aggregates in the  $i$ th fraction.

Higher MWD indicates higher proportions of macroaggregates. Geometric mean diameter (GMD) was calculated as

$$GMD = \exp \left( \frac{\sum W_i \ln X_i}{\sum W_i} \right) \quad (2.2)$$

where  $W_i$  is the weight of aggregates in size class  $i$  and  $X_i$  is the mean diameter of that size class. GMD estimates the size of the most frequent aggregate size class (Filho et al. 2002).

For isolation of microaggregates enclosed in macroaggregates, 5 g of macroaggregates (2.5 g each of large and small macroaggregates) were moistened with water (50 ml) and kept overnight at 4°C before isolation. Isolation was done according to the procedure of Six et al. (2000a). Macroaggregates were shaken with 50 glass beads over a 250 µm sieve where small macroaggregates were retained. Microaggregates were immediately flushed onto a 53 µm sieve (by steady water flowing through the isolation device) and later sieved to leave only the water-stable ones. Isolated silt and clay was obtained by drying 250 ml of the filtrate. Isolation was done only for plots where crop residue had been applied since they had better aggregation indices than plots without crop residue.

Laboratory analyses were conducted at the ICRAF soil laboratories in Nairobi. Soil bulk density was determined by using oven-dry weights (at 105°C for 48 hours) of soils sampled using 5 cm (diameter) by 5 cm (height) bulk density rings. Hot water-extractable carbon was determined on large macroaggregates for the 0-5 cm depth using a modified protocol (Ghani et al. 2003). Two grams soil was put into glass bottles and 25 ml of deionized water added before placing in an 80°C pre-heated water bath. Since

the interest was to capture the most labile carbon, the samples were left in the water bath for only 1 hour before cooling and pipetting. The carbon in the extract was determined by digesting with 0.0667M potassium dichromate ( $K_2Cr_2O_7$ ) to oxidize all the organic carbon and determine the amount of chromic ion ( $Cr^{3+}$ ) produced (ICRAF, 1995). In addition to the labile carbon, total soil carbon was also determined for the aggregates sizes from 0-5 cm and 5-20 cm depth using wet oxidation by acidified potassium dichromate. Intra-aggregate POM carbon was not analyzed, as isolates were too small. Measured bulk density was used in the calculation of the total carbon per hectare within the soil fractions.

All the weights of the different fractions were converted to percent of the initial weight before analyses. The data were analyzed using the SAS statistical package to obtain treatment effects and least square means, as well as Pearson's' correlation coefficients and their significance levels. Tillage, crop residue and cropping system were fixed effects, while replicates were treated as random effects in the procedure "Mixed". The statistical analysis for this split-split plot study was done according to the model of Federer and King (2007) for such a design. Pair-wise comparison of treatment means was done using the Tukey-Kramer method, which allows multiple comparison adjustment for the *p*-values and confidence limits for the differences of least square means.

## **2.3 Results**

### **2.3.1 Effects of tillage system**

Reduced tillage increased soil macroaggregation and thus decreased the portion of microaggregates as well as that of the free silt+clay soil fractions, as opposed to conventional tillage for all cropping systems (Table 2.1). For instance, on average, the contents of large macroaggregates were raised by 30-89% relative to the conventional tillage systems at the various depths; the increase was greatest at 10-15 cm soil depth where the tillage effect was significant ( $P<0.05$ ). At the same time, microaggregates were significantly ( $P<0.05$ ) lower in reduced tillage (by 23-28%) relative to conventional tillage at the three lower depths. Also, although only affected by tillage at the 5-10 cm depth, silt+clay content in all the four depths was in general 21-32% lower

in reduced tillage than in conventional tillage. Greater macroaggregates and the resulting lower microaggregates and silt+clay in reduced tillage can be attributed to reduced disruption of the aggregates by tillage operations as opposed to conventional tillage.

### **2.3.2 Effects of crop residue application**

Crop residue affected large macroaggregates ( $P<0.01$ ) and microaggregates ( $P=0.05$ ) only for the top 0-5 cm, because this was the depth where crop residue was concentrated. At this depth, there were on average 80% more large macroaggregates in treatments where crop residue was applied than in treatments without crop residue. In the reduced tillage system for example, the content of large macroaggregates was on average 102% higher with crop residue than without, while that of microaggregates was lower (by 16%). Besides bonding with encrusted microaggregates to form macroaggregates, crop residue likely also enhanced soil micro-organisms involved in soil aggregation. For soil depths below 5 cm, large macroaggregates were on average raised only slightly (by 3% to 19%) in treatments with crop residue compared to those without. Comparing the large macroaggregates in individual cropping systems for each tillage system revealed that at the top 0-5 cm, crop residue application in intercropping resulted in more than double the amount of large macroaggregates of treatments not receiving crop residue, but the effect in continuous maize and rotation systems was low and variable.

### **2.3.3 Effects of cropping system**

The type of cropping system affected ( $P<0.05$ ) soil aggregation in all the soil depths sampled because, unlike for the applied crop residue, crop root systems extend much deeper into the soil. Among the three systems tested, the highest proportions of macroaggregates and resulting lowest proportions of smaller aggregate sizes were found in the intercropping systems for both tillage treatments. At the various soil depths and regardless of tillage system, large macroaggregates were, for example, 20% to 104% and 44% to 150% higher in intercropping than continuous maize and soybean-maize rotation systems, respectively. At the same time, the intercropping system had 13% to 17% and 13% to 23% less microaggregate fraction, and 7% to 11% and 1% to 18%

lower silt+clay than continuous maize and rotation, respectively, depending on the depth. Usually, crop residue supply (amount and timing) is different in different cropping systems, and intercropping also tended to contain more water-soluble carbon than the rotation and continuous cereal systems (Figure 4.1). Macroaggregation was slightly raised in continuous maize relative to the soybean-maize rotation system, as macroaggregates formed during the legume phase likely disintegrated during the cereal phase, and vice versa.



Table 2.1. Effect of tillage, crop residue application and cropping system on soil aggregate classes in Nyabeda, western Kenya, in 2007

Tillage/ Cropping residue system	Large macroaggregates (>2mm)				SM (<2mm>250µm)				Microaggregates (>53<250µm)				Silt+clay (<53µm)																				
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D																	
----g 100g <sup>-1</sup> soil----																																	
CT-CR Continuous	3.8	cde	3.8	d	15.1	cde	26.3	bcd	37.9	ab	46.4	bcd	50.5	ab	46.0	abc	31.7	bc	27.3	abc	15.7	cd	13.8	abc	8.9	abc	7.5	ab	5.2	abc	3.6	ab	
	3.4	cde	8.0	abc	14.1	cde	31.0	abcd	41.5	ab	44.9	cd	48.3	bc	42.4	abc	28.0	bcd	24.2	abcd	18.1	abcd	13.0	abcd	9.2	abc	7.0	abc	7.0	a	3.6	ab	
	Rotation	2.0	e	2.1	d	10.2	de	24.6	bcd	32.8	c	44.4	cd	50.6	ab	48.0	ab	38.1	a	29.4	a	20.4	ab	13.7	abc	10.7	a	8.4	a	5.1	abc	2.2	cd
CT+CR Continuous	2.7	cde	5.0	bcd	8.2	e	21.7	cd	37.7	abc	43.1	d	51.0	ab	49.2	a	31.5	bc	28.4	ab	21.8	a	15.5	a	9.8	abc	8.7	a	6.3	ab	2.8	bcd	
	8.3	b	9.8	a	13.2	cde	35.9	abc	41.5	ab	45.9	bcd	51.0	ab	40.2	bc	23.7	de	22.4	bcd	16.9	bcd	10.7	bcde	7.8	bc	6.5	bcd	5.4	abc	2.5	bcd	
	Rotation	2.7	de	4.2	d	6.9	e	19.0	d	37.1	bc	45.3	cd	55.5	a	49.2	a	32.5	b	28.2	ab	18.7	abc	15.2	ab	10.0	ab	7.5	ab	4.3	bcd	3.9	a
RT-CR Continuous	3.6	cde	9.2	ab	18.4	bcd	36.1	ab	40.3	ab	51.1	abc	48.4	bc	42.2	abc	29.1	bcd	19.6	d	15.3	cd	9.6	cde	8.3	abc	4.5	d	3.9	cd	1.8	cd	
	5.2	bcde	9.1	ab	24.2	ab	36.0	abc	41.0	ab	49.6	abcd	43.9	cd	42.8	abc	27.0	cd	20.9	cd	14.1	de	9.0	de	8.5	abc	5.7	bcd	3.3	cd	2.2	cd	
	Rotation	4.3	cde	4.0	d	13.9	cde	25.8	bcd	42.3	ab	54.4	a	52.0	ab	49.2	a	27.1	cd	20.7	cd	15.9	cd	11.7	abcde	8.5	abc	5.0	cd	4.9	abc	2.7	bcd
RT+CR Continuous	6.0	bc	8.1	abc	18.5	bcd	35.5	abc	43.4	a	53.0	ab	47.3	bc	43.3	abc	24.3	de	22.9	abcd	16.2	bcd	9.5	cde	7.0	c	5.5	bcd	4.1	bcd	2.2	cd	
	16.0	a	11.2	a	31.4	a	41.4	a	40.6	ab	51.5	abc	40.7	d	38.9	c	19.9	e	17.6	d	10.7	e	7.6	e	4.6	e	5.2	cd	2.4	d	1.5	d	
	Rotation	4.5	cde	4.9	cd	21.3	bc	30.8	abcd	43.3	a	52.7	ab	48.2	bc	45.8	abc	25.4	d	22.0	bcd	13.4	de	11.4	abcde	7.6	bc	6.1	bcd	3.9	cd	2.9	abc
SE	1.2		1.4		2.9		5.8		2.2		2.7		2.0		3.2		2.2		2.8		1.6		1.7		1.0		0.8		0.8		0.4		
Tillage					*												*			*		*		*		*			*				
CR	**																*																
CS	***		***		**		**		**		***		***		**		***		*		*		*		*		*						
Tillage*CS										***							*													*			
CR*CS	***																					*								***		*	
Tillage*CR*CS																																*	

SM=small macroaggregates, A=0-5 cm depth, B=5-10 cm depth, C=10-15 cm depth and D=15-20 cm depth. CR=Crop residue applied at 2 t ha<sup>-1</sup>, CT=conventional tillage, RT=reduced tillage, CS=Cropping system, SE= standard error. Values in the same column followed by the same letter are not significantly different at P=0.05. \*significant effect at P<0.05, \*\*significant effect at P<0.01, \*\*\*significant effect at P<0.001

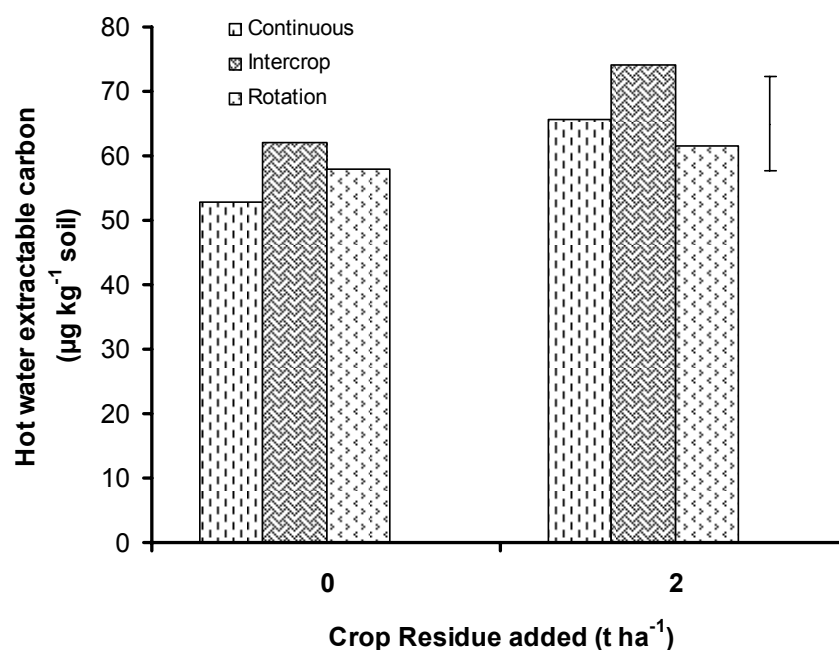


Figure 2.1. Effect of cropping system and crop residue on hot water-extractable carbon from large macroaggregates at 0-5 cm depth in Nyabeda, western Kenya

#### 2.3.4 Mean weight diameter (MWD) and geometric mean diameter (GMD)

Aggregation indices like MWD and GMD are a simplified means to evaluate the degree of aggregation under different land-use systems. Increased soil macroaggregation observed in reduced tillage, positive effects of crop residue application as well as contribution of legume-cereal intercropping resulted in significantly higher aggregate MWD and GMD (Table 2.2). The tillage effect was significant at the top three soil depths ( $P < 0.05$ ) with 19% to 35% greater MWD and 30% to 42% greater GMD in reduced tillage than in conventional tillage. As with soil aggregation, the crop residue effect was significant at the 0-5 cm soil depth only ( $P < 0.01$ ), with treatments with crop residue having 23% greater MWD and 32% greater GMD than treatments without crop residue, regardless of tillage system. The effect of cropping system was, however, significant even at the lowest soil depth (15-20 cm;  $P < 0.01$ ). In general and at the various soil depths, intercropping had 12% to 42% greater MWD and similarly greater GMD than in both continuous maize and soybean-maize rotation systems. Pooled data for the 0-20 cm depth also showed higher aggregation indices in reduced than in conventional tillage and again higher indices in the maize-soybean intercropping system than in rotation and continuous maize (data not shown).

Table 2.2. Effect of tillage, crop residue application and cropping system on aggregate mean weight diameter (MWD) and geometric mean diameter (GMD) in Nyabeda, western Kenya, in 2007

Tillage	CR	Cropping system	Mean Weight Diameter -----mm-----					Geometric Mean Diameter -----mm-----				
			0-5cm	5-10cm	10-15cm	15-20cm		0-5cm	5-10cm	10-15cm	15-20cm	
CT	-	Continuous	0.92 <sup>def</sup>	1.02 <sup>ef</sup>	1.70 <sup>bcd</sup>	2.20 <sup>bcd</sup>		0.41 <sup>de</sup>	0.52 <sup>cd</sup>	0.92 <sup>bcd</sup>	1.24 <sup>cd</sup>	
	-	Intercrop	0.95 <sup>cde</sup>	1.25 <sup>abcde</sup>	1.59 <sup>cde</sup>	2.39 <sup>abcd</sup>		0.44 <sup>cde</sup>	0.62 <sup>bcd</sup>	0.74 <sup>de</sup>	1.38 <sup>bcd</sup>	
	-	Rotation	0.73 <sup>f</sup>	0.89 <sup>f</sup>	1.43 <sup>de</sup>	2.15 <sup>bcd</sup>		0.31 <sup>f</sup>	0.46 <sup>e</sup>	0.75 <sup>de</sup>	1.34 <sup>cd</sup>	
	+	Continuous	0.86 <sup>ef</sup>	1.05 <sup>def</sup>	1.31 <sup>e</sup>	2.00 <sup>cd</sup>		0.38 <sup>ef</sup>	0.49 <sup>de</sup>	0.68 <sup>e</sup>	1.14 <sup>d</sup>	
	+	Intercrop	1.25 <sup>b</sup>	1.37 <sup>abc</sup>	1.60 <sup>cde</sup>	2.65 <sup>abc</sup>		0.56 <sup>b</sup>	0.67 <sup>abc</sup>	0.86 <sup>cde</sup>	1.63 <sup>abc</sup>	
	+	Rotation	0.85 <sup>ef</sup>	1.03 <sup>ef</sup>	1.34 <sup>e</sup>	1.87 <sup>d</sup>		0.37 <sup>ef</sup>	0.51 <sup>de</sup>	0.78 <sup>de</sup>	1.05 <sup>d</sup>	
RT		<i>Average</i>	0.93	1.10	1.50	2.21		0.41	0.55	0.79	1.30	
	-	Continuous	0.96 <sup>cde</sup>	1.41 <sup>ab</sup>	1.87 <sup>bc</sup>	2.67 <sup>ab</sup>		0.44 <sup>de</sup>	0.77 <sup>ab</sup>	1.04 <sup>bcd</sup>	1.71 <sup>ab</sup>	
	-	Intercrop	1.06 <sup>bcd</sup>	1.37 <sup>abc</sup>	2.15 <sup>ab</sup>	2.66 <sup>abc</sup>		0.48 <sup>bcd</sup>	0.71 <sup>ab</sup>	1.19 <sup>ab</sup>	1.68 <sup>abc</sup>	
	-	Rotation	1.02 <sup>cde</sup>	1.16 <sup>cde</sup>	1.66 <sup>cde</sup>	2.22 <sup>bcd</sup>		0.47 <sup>bcd</sup>	0.67 <sup>bc</sup>	0.91 <sup>bcd</sup>	1.34 <sup>cd</sup>	
	+	Continuous	1.16 <sup>bc</sup>	1.31 <sup>abcd</sup>	1.85 <sup>bcd</sup>	2.64 <sup>abc</sup>		0.55 <sup>bc</sup>	0.70 <sup>ab</sup>	1.01 <sup>bcd</sup>	1.66 <sup>abc</sup>	
	+	Intercrop	1.71 <sup>a</sup>	1.52 <sup>a</sup>	2.52 <sup>a</sup>	2.92 <sup>a</sup>		0.82 <sup>a</sup>	0.83 <sup>a</sup>	1.49 <sup>a</sup>	1.96 <sup>a</sup>	
SE	+	Rotation	1.06 <sup>bcd</sup>	1.17 <sup>bcd</sup>	2.01 <sup>bc</sup>	2.41 <sup>abcd</sup>		0.51 <sup>bcd</sup>	0.64 <sup>bcd</sup>	1.17 <sup>bc</sup>	1.46 <sup>bcd</sup>	
		<i>Average</i>	1.16	1.32	2.01	2.59		0.55	0.72	1.14	1.64	
			0.078	0.102	0.167	0.237		0.036	0.063	0.119	0.194	
			*	*	*	*		*	*	*	*	
			**	**	**	**		**	**	**	**	
			***	***	***	***		***	***	***	***	
Tillage*CR			***	***	*	**		**	**	**	**	
			***	***	*	**		**	**	**	**	
			***	***	*	**		**	**	**	**	
			***	***	*	**		**	**	**	**	
			***	***	*	**		**	**	**	**	
			***	***	*	**		**	**	**	**	

Values in the same column followed by the same letter are not significantly different at P=0.05. CR=Crop residue applied at 2 t ha<sup>-1</sup>, CT=conventional tillage, RT=reduced tillage, CS=Cropping system, SE= standard error, \*significant effect at P<0.05, \*\*significant effect at P<0.01, \*\*\*significant effect at P<0.001

### **2.3.5 Clay, silt and microaggregates within macroaggregates**

Separating the macroaggregates (large + small) into microaggregates and free silt and clay particles revealed first that as soil depth increased, the portion of free silt+clay particles increased at the expense of microaggregates formed within the macroaggregates ( $P < 0.05$ ; Table 2.3). The increase was particularly noticeable moving from 5-10 cm to 10-15 cm depths, with a 21% increase in isolated silt+clay in conventional tillage and 24% in reduced tillage, perhaps because soil micro-organisms involved in aggregate stabilization usually decrease with depth. Secondly, and surprisingly, the isolation resulted to average 15% to 18% more free silt+clay particles (and 1% to 8% less microaggregates, depending on depth) held inside the macroaggregates in reduced than in conventional tillage, even though the macroaggregates had been greater in reduced than in conventional tillage as earlier reported. In line with this, there was positive and significant ( $P < 0.05$ ) correlations between whole-soil macroaggregates and isolated silt+clay in the rotation ( $R^2 = 0.40$ ), intercropping ( $R^2 = 0.44$ ) and continuous maize treatments ( $R^2 = 0.40$ ; also Figure 2.2). These results show that whenever higher macroaggregation was observed, it was accompanied by a higher proportion of macroaggregates within which silt and clay are not well stabilized into microaggregates. Intra-aggregate particulate organic matter (iPOM) recovered from macroaggregates varied from 1 to 5 g 100g<sup>-1</sup> macroaggregate soil; it decreased with depth ( $P < 0.05$ ). For example, iPOM for the 10-15 cm depth was lower than that for the 0-5 cm depth by 19% to 74% in conventional tillage and by 15% to 21% in reduced tillage, which can be attributed to the usually decreased soil organic matter at lower soil depths. The decrease was consistent with the decreasing isolated microaggregates and increasing silt+clay at lower depth.

Table 2.3. Silt and clay, microaggregates and intra-aggregate particulate organic matter (iPOM) isolated from whole soil macroaggregates for different tillage and cropping systems in western Kenya, in 2007

Tillage	Cropping system	Silt+clay			Microaggregates			iPOM		
		---(g 100g <sup>-1</sup> soil)---			---(g 100g <sup>-1</sup> soil)---			---(g 100g <sup>-1</sup> soil)---		
		0-5 cm	5-10 cm	10-15 cm	0-5 cm	5-10 cm	10-15 cm	0-5 cm	5-10 cm	10-15 cm
Conventional tillage	Continuous	29.1 <sup>ab</sup>	27.0 <sup>ab</sup>	32.0 <sup>a</sup>	63.0 <sup>a</sup>	66.5 <sup>a</sup>	60.6 <sup>a</sup>	2.6 <sup>a</sup>	2.1 <sup>a</sup>	2.1 <sup>ab</sup>
Conventional tillage	Intercrop	29.6 <sup>ab</sup>	30.0 <sup>ab</sup>	35.9 <sup>a</sup>	63.9 <sup>a</sup>	65.5 <sup>a</sup>	60.2 <sup>a</sup>	3.5 <sup>ab</sup>	2.0 <sup>a</sup>	2.8 <sup>b</sup>
Conventional tillage	Rotation	24.5 <sup>a</sup>	25.9 <sup>a</sup>	35.0 <sup>a</sup>	67.0 <sup>a</sup>	65.5 <sup>a</sup>	61.8 <sup>a</sup>	5.1 <sup>b</sup>	3.6 <sup>a</sup>	1.3 <sup>a</sup>
	<i>Average</i>	27.7	27.6	34.3	64.6	65.8	60.9	3.7	2.6	2.1
Reduced tillage	Continuous	31.8 <sup>b</sup>	31.7 <sup>ab</sup>	39.9 <sup>a</sup>	63.2 <sup>a</sup>	61.9 <sup>a</sup>	56.1 <sup>a</sup>	2.8 <sup>a</sup>	3.1 <sup>a</sup>	2.2 <sup>ab</sup>
Reduced tillage	Intercrop	34.4 <sup>b</sup>	30.8 <sup>ab</sup>	39.3 <sup>a</sup>	63.2 <sup>a</sup>	63.0 <sup>a</sup>	55.4 <sup>a</sup>	2.0 <sup>a</sup>	2.5 <sup>a</sup>	1.7 <sup>ab</sup>
Reduced tillage	Rotation	30.1 <sup>ab</sup>	35.2 <sup>b</sup>	39.3 <sup>a</sup>	65.4 <sup>a</sup>	59.4 <sup>a</sup>	56.9 <sup>a</sup>	2.3 <sup>a</sup>	2.5 <sup>a</sup>	1.9 <sup>ab</sup>
	<i>Average</i>	32.1	32.6	39.5	63.9	61.4	56.1	2.4	2.7	1.9
SED		1.92	2.88	3.15	2.94	2.73	2.76	0.74	0.83	0.43
SED <sup>#</sup>		1.38			1.72			0.31		
Depth effect		***			**			*		

Values in the same column followed by the same letter are not significantly different at P=0.05, SED=standard error of differences of means, All plots had added crop residue at 2 t ha<sup>-1</sup>. <sup>#</sup> for comparing means across depths, \*significant effect at P<0.05, \*\*significant effect at P<0.01, \*\*\*significant effect at P<0.001

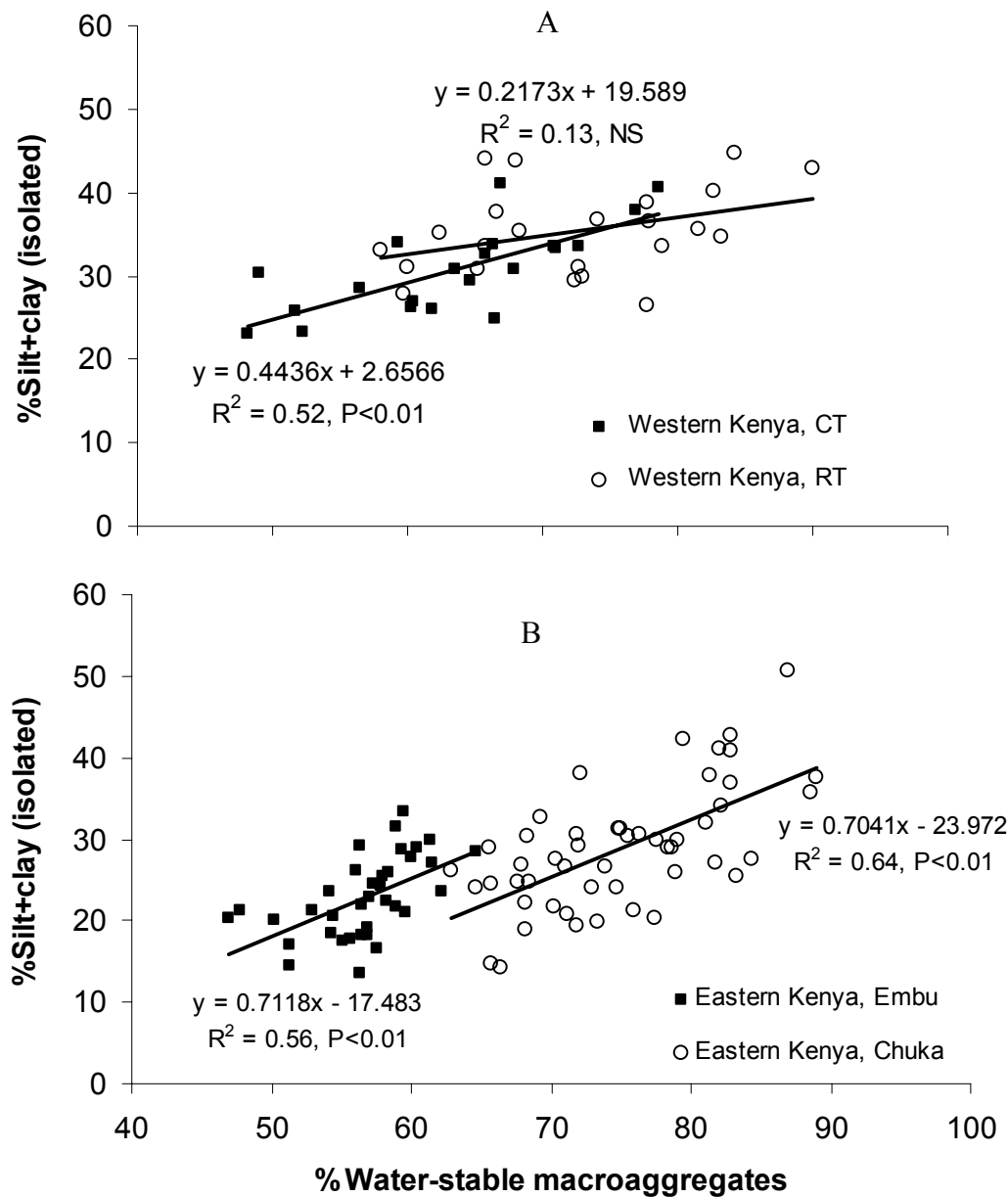


Figure 2.2. Relationship of water-stable macroaggregates and silt+clay isolated from macroaggregates in (A) Nyabeda-western Kenya (n=27 for RT and 27 for CT) and, (B) Embu (n=36) and Chuka (n=50) in eastern Kenya, CT=conventional tillage, RT=reduced tillage. Unpublished data for Chuka and Embu was obtained from Agnes Kavoo

### 2.3.6 Soil carbon

Changes in bulk densities must be considered when evaluating total carbon (C) storage within soil depth intervals. In our study, bulk density at the 5-20 cm depth was slightly elevated in reduced tillage compared with the conventional tillage treatments (Table 2.4), probably because of the lack of continued soil loosening by tillage.

Total carbon stocks varied between 8.1 and 9.2 t C ha<sup>-1</sup> in the top 0-5 cm, and 26.6 and 29.8 t C ha<sup>-1</sup> in the 5-20 cm depth, with no differences due to tillage, crop residue or cropping system (data not shown). Also, a low amount of iPOM was observed within the macroaggregates as shown earlier in Table 2.3. As a result, there was no drastic increase in percent carbon with aggregate size (microaggregates (1.49%) = small macroaggregates (1.54%) < large macroaggregates (1.69%) < Silt+clay (1.82%) for treatment with crop residue and in the top 5 cm depth). Also, only low correlations between the aggregation indices and % SOC content in the aggregate fractions ( $R^2 > 0 < 0.25$ ) were observed in the top 5 cm (data not shown). There were, however, differences in carbon distribution among aggregate sizes (Table 2.4). Due to greater aggregation, total soil C stocks associated with >2mm aggregates were on average 76% and 41% higher for the reduced tillage than for the conventional tillage treatments in 0-5 cm and 5-20 cm depths, respectively. This resulted in lower (by 35%) microaggregate-associated carbon in reduced than in conventional tillage ( $P < 0.05$ ) in the 5-20 cm depth. As expected, carbon stocks in the large macroaggregate soil fraction in the top 0-5 cm were increased by an average of 80% with crop residue ( $P < 0.05$ ) compared to without crop residue. Cropping system affected carbon stocks ( $P < 0.001$ ) in both 0-5 cm and 5-20 cm depths, and with crop residue application for example, carbon associated with large macroaggregates was in the order intercropping > continuous maize = rotation system.

Table 2.4 Effect of tillage, crop residue application and cropping system on soil bulk density and carbon stocks in different soil aggregate fractions and soil depths in Nyabeda, western Kenya, in 2007

Tillage	CR	CS	Bulk density ---g cm <sup>-3</sup> ---			LM	SM	Mi	Silt+clay -----t C ha <sup>-1</sup> -----			SM	Mi	Silt+clay								
			0-5 cm			5-20 cm			0-5 cm depth			5-20 cm depth										
CT	-	Continuous	1.18	bc	1.17	ab	0.36	ab	4.44	bc	3.49	de	1.00	c	4.73	ab	15.7	ab	6.22	cdef	1.90	b
	-	Intercrop	1.13	abc	1.25	c	0.42	ab	4.67	bc	2.98	cd	0.96	bc	5.89	abc	15.4	ab	6.47	def	2.02	b
	-	Rotation	1.13	abc	1.17	abc	0.26	ab	3.48	a	3.92	e	0.96	bc	3.67	a	15.6	ab	6.77	ef	1.90	b
	+	Continuous	1.10	abc	1.18	abc	0.37	ab	4.05	ab	3.08	cd	0.96	bc	3.59	a	15.7	ab	7.36	f	2.00	b
	+	Intercrop	1.06	a	1.21	abc	0.89	bc	4.39	bc	2.29	ab	0.73	ab	6.42	bc	15.0	ab	5.24	abcd	1.70	ab
	+	Rotation	1.08	ab	1.16	a	0.25	a	4.13	abc	3.43	de	1.02	c	3.58	a	15.2	ab	6.28	def	1.72	ab
RT		<i>average</i>	1.11		1.19		0.42		4.19		3.20		0.93		4.65		15.43		6.39		1.88	
	-	Continuous	1.15	abc	1.22	abc	0.37	ab	4.09	ab	2.88	bcd	0.81	bc	7.05	cd	14.1	a	4.47	ab	1.14	a
	-	Intercrop	1.15	abc	1.18	abc	0.59	abc	4.90	c	2.84	bcd	0.91	bc	7.27	cd	14.1	a	4.57	ab	1.65	ab
	-	Rotation	1.12	abc	1.23	bc	0.49	abc	4.74	c	2.87	bcd	0.86	bc	4.75	ab	17.0	b	5.53	bcd	1.63	ab
	+	Continuous	1.19	c	1.22	bc	0.71	bc	4.97	c	2.52	abc	0.76	abc	6.43	bc	14.8	ab	4.59	abc	1.46	ab
	+	Intercrop	1.10	abc	1.23	bc	1.72	d	4.59	bc	2.02	a	0.49	a	9.24	d	13.9	a	3.88	a	1.29	a
SE	+	Rotation	1.18	c	1.22	bc	0.56	abc	4.99	c	2.53	abc	0.84	bc	4.61	ab	16.0	ab	5.32	bcd	1.90	b
		<i>average</i>	1.15		1.22		0.74		4.71		2.61		0.78		6.56		15.00		4.73		1.51	
			0.034		0.023		0.161		0.353		0.263		0.117		0.955		0.851		0.589		0.218	
Tillage																			*			
CR							*				*											
CS							***				**				***					*		
Tillage x CS					*				*													
CR x CS							**															

Values in the same column followed by the same letter are not significantly different at P=0.05. \*significant effect at P<0.05, \*\* significant effect at P<0.01, \*\*\* significant effect at P<0.001, LM= Large macroaggregates, SM= Large macroaggregates, Mi= Microaggregates, CR=crop residue applied at 2 t ha<sup>-1</sup>, CT=conventional tillage, RT=reduced tillage, CS=cropping system, SE= standard error



## **2.4 Discussion**

### **2.4.1 Effects of tillage system**

Greater soil macroaggregation in reduced tillage as opposed to conventional tillage reported in this study is consistent with the findings of other researchers (Peixoto et al. 2006; Six et al. 2000b). For example, higher proportions of macroaggregates under reduced tillage as opposed to tilled plots have been reported under Brazilian Ferralsols (Filho et al. 2002; Pinheiro et al. 2004). Disturbing the soil through conventional tillage, as commonly done in western Kenya, is able to break the bonds between the aggregates of the Ferralsols, while reduced tillage obviously better preserves the aggregates. The disturbance can also accelerate decomposition of organic matter (Zotarelli et al. 2007), and loss of such organic matter leads to a substantial loss of macroaggregates (Oades 1984). Besides, fungi, whose hyphae play a significant role in enmeshment of aggregates (Oades 1984), are more abundant in reduced tillage systems (Beare et al. 1993; Degens et al. 1996), and large macroaggregates can be reduced by up to 40% in the absence of fungi (Beare et al. 1997). The high proportions of macroaggregates (up to 85% of total soil) found in this study are in agreement with other studies conducted in similar climates and soil type (Lehmann et al. 2001; Madari et al. 2005). Such a high proportion of macroaggregates is expected in Ferralsols due to the presence of iron and aluminum oxides that participate in formation and stabilization of aggregates (Six et al. 2000b).

Separating the macroaggregates (large + small) into microaggregates and free silt and clay particles provides deeper insights into the mechanisms of aggregate formation than indicated from mere size fractionation (Six et al. 2000a). Our results show that the microaggregates within macroaggregates were more in the very top soils than in the deeper ones. Decreased microaggregates within macroaggregates at lower depth may be related to microbial abundance, which becomes scarcer with increasing soil depth (Doran 1980), i.e., micro-organisms may add less to microaggregate formation and stabilization within the macroaggregates at lower depth in this tropical environment.

Although reduced tillage was able to increase the amount of macroaggregation, in tandem with our hypothesis, it did not yet also promote the formation of microaggregates within the macroaggregates as in the conventional tillage

treatments. In Brazil, and in a 15-year old experiment dominated by 1:1 kaolinite as in our case, no differences in microaggregates within macroaggregates between reduced- and conventional tillage were observed (Denef et al. 2004). However, more microaggregates enclosed within macroaggregates in reduced tillage systems compared with conventional tillage ones have been reported for Illites and soils of mixed mineralogy and were attributed to longer stabilization periods without disturbance (Denef et al. 2004; Six et al. 2000a). In the case of the current study, it seems reasonable to assume that the additional macroaggregates in the reduced tillage treatments exhibited a lower stability and may re-degrade faster in soil. In other words, this finding indicates that at least some of the microaggregates of the conventional tillage treatments were additionally stabilized inside the larger macroaggregates. Also, it may be assumed that the available period of stabilization at the study site was not long enough, i.e., exposure to only 5 years of different tillage compared with 30 years in the study of Six et al. (2000a).

#### **2.4.2 Effects of crop residue application**

Kong et al. (2005) reported a significant linear relationship between aggregate stability and soil carbon for soils under different Mediterranean cropping systems. Increased soil macroaggregation when crop residue was applied may be explained by microaggregates encrusting large POM from the applied crop residues, thus reducing free microaggregates and increasing large macroaggregates (Six et al. 2000a). Also, applied crop residue affects microbial diversity, composition and abundance and can lead to increased aggregation activity because micro-organisms secrete extracellular polysaccharides that stabilize soil aggregates (Beare et al. 1997). Lower decomposition rates of surface-placed residue (longer persistence) than buried residue may explain the tendency of greater macroaggregation when crop residue was added in reduced- than in conventional tillage in our study. The advantage of surface application of crop residue has been demonstrated in greater crop-derived carbon in macroaggregates when residue is surface-applied than when incorporated (Olchin et al. 2008), perhaps an indication of greater carbon losses in the case of incorporated residue.

The aggregate hierarchy theory is evident for Alfisols and Mollisols, and the mechanism of microaggregate formation within macroaggregates has been observed in

such soils but not in Ferralsols (Six et al. 2004; Six et al. 2000b). For example, aggregate hierarchy was absent in a study by Zotarelli et al. (2005), because the soils were dominated by 1:1 kaolinite clays and oxides. Our results show that, in addition to more microaggregates enclosed with macroaggregates in surface than in lower soil strata, organic carbon was higher in large macroaggregates than in both small macroaggregates and microaggregates, demonstrating that SOM binds microaggregates into macroaggregates. However, the less pronounced aggregate hierarchy in our study (as opposed to the results in Six et al. (2000b) can be attributed to the presence of oxides, which play a dominant role in aggregation within Ferralsols. We suggest, therefore, that despite the fact that the soils were dominated by inorganic (oxidic) bonding agents, managing soil organic matter remains of great importance for the structural stability of the very top soil.

#### **2.4.3 Effects of cropping system**

Crop residue supply (amount and timing) is different in different cropping systems. Probably, the continuous presence of a legume in the intercropping system favored the stability or re-formation of macroaggregates or both, possibly via its root residues and organic exudates released by the legume (Latif et al. 1992), by differences in its associated microbial community (this study) or simply via differences in root architecture and hence deposition of organic carbon (Degens 1997). In general, higher root densities, which are known to be correlated with soil aggregation (Haynes et al. 1991), may be expected for the intercropping system due to its higher plant density (maize plus soybean) relative to the other systems. Intercropping also tended to contain more water-soluble carbon, which has also been shown to correlate positively with soil aggregation (Degens 1997). Nevertheless, under rotation, the macroaggregates formed during the legume phase likely break up after the legume crop is removed, leading to increased microaggregates and silt and clay fractions as observed in the top 0-5 cm depth in this study. Non-significant effects of rotations on soil aggregation in Ferralsols, and in climatic conditions similar to ours, have also been found in Brazil (Filho et al. 2002; Madari et al. 2005). While the results in the intercropping system support our hypothesis of higher macroaggregation in legume-based systems compared to

continuous cereal, separating the crop components in time (rotation) did thus not seem to benefit macroaggregate formation over the continuous cereal monocropping system.

The higher aggregation index for the intercropping system could contribute to the higher land equivalence ratios in intercropping compared to rotation and continuous maize systems reported in several studies (Bationo 2008; Diangar et al. 2004; Karikari et al. 1999; Niringiye et al. 2005; Worku 2004). Cumulative maize yield from two successive cropping seasons in intercropping ( $4.5 \text{ t ha}^{-1}$  grain) was comparable to the seasonal average in rotation ( $4.7 \text{ t ha}^{-1}$ ). However, with maize, although the rotation achieved higher yields than the continuous system (average grain yield in continuous maize system was only  $3.8 \text{ t ha}^{-1}$ ), its aggregation index is not better than that of the latter. This implies that where crop rotation is being practiced, aggregation indices may not reflect well the fertility status of soil, especially because the indices depend on crop cycle, and because there are several bio-physical and chemical factors determining soil fertility and resultant crop yield.

#### **2.4.4 Soil carbon**

In principle, a better aggregation provides the chance of improving soil tilth at larger porosity and higher SOM content, as intact soil aggregates provide additional capacity to the soil to sequester carbon. Studies have shown greater carbon in reduced tillage compared to conventional tillage systems (Denef et al. 2004; Zotarelli et al. 2007), and attributed it to enhanced carbon stabilization in microaggregates within macroaggregates due to slower turnover of the macroaggregates in reduced tillage systems (Zotarelli et al. 2007). Lack of tillage, crop residue or cropping system effects on total carbon stocks may be related to the fact that aggregation in oxidic soils such as those in western Kenya is not only due to SOM but also due to electrostatic attractions between oxides and 1:1 kaolinite clays (Six et al. 2000b). Within the short period of different land use in our study, preservation of SOM within large aggregates under reduced tillage is still foiled by lower stability of the macroaggregates. This may explain why we failed to detect a higher total carbon stock under a better macroaggregation regime. Only after much longer than 5 years of management (Six et al., 2000a), formation of microaggregates within the macroaggregates of the reduced tillage treatments may have been developed enough to also positively affect carbon

sequestration, and thus contribute to different treatment effects on macroaggregate iPOM composition that were not evident yet. The results also indicate that more studies that take into account carbon sequestration in Ferralsols and within a longer time horizon are needed, and one should refrain from generalizing too easily results on conservation agriculture between sites and with too short data sets. The significant crop residue and cropping system effects on carbon stocks in the large macroaggregate soil fraction in the top 0-5 cm depth reflects differences in organic matter input, and are an indication that good management can ultimately result in greater carbon sequestration in these Ferralsols. The increased carbon in large as opposed to small macroaggregates and microaggregates observed in this study is also an indication of initial processes towards greater carbon sequestration, which may manifest also in aggregated carbon stocks after more seasons of practice. We conclude that carbon sequestration in Ferralsols such as in western Kenya could take longer than 5 years to come into effect due to iron and aluminum oxides and dominating 1:1 kaolinite clay minerals that also contribute to soil aggregation, in addition to SOM.

## **2.5 Conclusions**

Practicing reduced tillage resulted in 30% to 89% more macroaggregates and 19% to 42% greater aggregation indices compared to conventional tillage, supporting the hypothesis of greater macroaggregation in reduced than in conventional tillage. However, the hypothesis of greater microaggregates protected within macroaggregates in reduced tillage was not supported since reduced tillage resulted in 1-8% less isolated microaggregates and 15-18% more free silt+clay than conventional tillage. Among the cropping systems, intercropping increased large macroaggregates by 20-150% and aggregation indices by 12-42% compared to both continuous maize and soybean-maize rotation systems. Thus, aggregation in intercropping support the hypothesis that cropping systems involving legumes increase aggregation over continuous cereal systems, but aggregation in rotation did not support this hypothesis. Applied crop residue increased soil aggregation by 80% in the top soil layer and by 3-19% in the other layers compared to plots without crop residue. Combining reduced tillage and crop residue application as well as legume-maize intercropping is one of the best strategies to improve soil aggregation.

### **3 DIVERSITY OF BACTERIA AND FUNGI IN REDUCED TILLAGE SYSTEMS IN SUB-HUMID AND ARID ZONES IN KENYA**

#### **3.1 Introduction**

Tillage, crop residue management and cropping systems all affect soil microbial diversity. Reduced tillage increases enzyme activity, soil macroaggregation, microbial biomass and colonization of soil by microbes when compared to conventional tillage systems (Nsabimana et al. 2004; Six et al. 2006). For example, greater abundance of fungi has been reported in reduced than conventional tillage in a sandy clay loam in the USA (Beare et al. 1993). Reducing tillage leads to decreased loss rates of organic matter relative to conventional tillage systems (Zotarelli et al. 2007), which can affect soil microbes. Although reduced tillage has been practiced in Africa for some time now, little systematic research of its effects on microbial diversity has been undertaken.

Particularly, crop residue management is a central component of conservation agriculture. Besides driving soil moisture and micro-climate, crop residue mulch provides a microbial substrate and carbon source that affects microbial growth (Alden et al. 2001), composition and diversity (Six et al. 2006). For example, the abundance of Acidobacteria decreased while that of  $\beta$ -Proteobacteria and Bacterioidetes increased with increasing carbon (Fierer et al. 2007). It requires readily available residues, but farming in most of sub-Saharan Africa is characterized by low availability of organic resources as, typically, little of the previous season's crop residue is left on the fields (Bationo et al. 2007). The relative effect of such crop residue on bacterial and fungal diversity in soils of different tillage systems in the agro-climatic zones of eastern Africa is not well studied.

Different crop species can also have significant effects on the diversity of micro-organisms, especially in the rhizosphere (Bremer et al. 2007; Morris et al. 2002). For example, soil denitrifier communities were different in soils under different non-leguminous grassland plant species in Germany (Bremer et al. 2007). Fierer et al. (2007) showed that some micro-organisms have copiotrophic (*r*-selected) or oligotrophic (*K*-selected) tendencies, and increase in relative abundance in the presence of high and low carbon availability, respectively. In the USA, soil fungal biomass and diversity

increased when supplemented with diverse carbon sources in a study demonstrating the role of root exudates in shaping soil fungal community (Broeckling et al. 2008). In Kenya, the three common cropping systems, namely maize-maize, maize-legume rotation and maize/legume intercropping, present different spatial and temporal arrangements of plant species that could affect microbial diversity. Although it is generally agreed that diversity of organic resources increases diversity of soil micro-organisms (Broeckling et al. 2008), relatively less attention has been given to intercropping systems than to pure crop stands grown under rotation. Thus, the extent to which intercropping affects microbial diversity relative to continuous monocropping and rotation remains largely unknown. Continuous supply of legume residues, both as surface litter-fall and belowground root dieback in intercropping could result in higher microbial diversity than under continuous monocropping and rotation.

Previous research has focused on the role of specific soil micro-organisms in the aggregation of soil particles (Amellal et al. 1998). Abundance of specific microbial species increases aggregate stability through production of extracellular compounds (Alami et al. 2000; Metting 1986). However, it is still not clear how microbial diversity affects soil aggregation. Development of advanced molecular techniques based on rDNA, such as polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), has progressed rapidly, enabling the analysis of the diversity and composition of microbial communities (Øvreås and Torsvik 1998). The techniques offer opportunities for soil microbial fingerprinting and assessment of diversity among different soils and management practices. But relating such genomic data with soil aggregation indices is rarely done.

Soil type, physical and chemical properties as well as ecosystem type (Fierer and Jackson 2006; Girvan et al. 2003) also affect richness and diversity of microbial communities. This study was therefore conducted with soil samples from contrasting sites in Kenya to determine the effects of tillage systems, residue management and cropping systems on composition and diversity of soil bacteria and fungi, and to identify how the diversity relates to soil parameters.

The hypotheses were that:

1. Reduced tillage and crop residue application both affect composition and increase soil microbial diversity compared to systems that are conventionally tilled and without crop residue,
2. Cropping systems involving different crops grown concurrently, such as in intercropping, have higher microbial diversity than monocrop systems such as in continuous or rotation systems, and
3. Soil aggregation increases with diversity of fungi and bacteria community composition.

### **3.2 Materials and methods**

This study was conducted in two agro-ecological zones: a semi-arid zone (Machang'a in eastern Kenya) and a sub-humid zone (Matayos and Nyabeda in western Kenya). The experiment in the semi-arid site was a randomized complete block design with 3 tillage systems (conventional tillage, no-till and tied-ridging) each with two organic applications (2 t ha<sup>-1</sup> manure applied alone and, 1 t ha<sup>-1</sup> manure plus 1 t ha<sup>-1</sup> crop residue). The experiments in the sub-humid zone were designed as split-split plot with tillage (conventional and reduced tillage), crop residue (0 and 2 t ha<sup>-1</sup> maize stover) and cropping systems (continuous maize, maize soybean intercropping and rotation) as the main, split and split-split plots respectively (section 1.5).

Fresh soil samples were collected in April 2008 after ten seasons of experimentation in Nyabeda, western Kenya, at two weeks after planting. These were soil samples from 0-10 cm depth where most of the microbial diversity and activity was assumed to be highest (Agnelli et al. 2004). The samples were delivered to the laboratory the same day, kept overnight at 4°C and 10 g soil re-sampled and kept at -20°C until DNA extraction and analysis. Dry soils from 0-15 cm depth collected in March 2008 and September 2007 were used for Matayos (western Kenya) and Machang'a (eastern Kenya), respectively, after incubation at a controlled temperature of 20°C for 21 days to reactivate micro-organisms. All soil samples were taken at five points within a plot and thoroughly mixed so that the microbial samples were representative of the treatments. For each of the analysis, three replicates were used (except for Matayos with two replicates). To allow direct comparisons, all treatments



within a replicate were treated in the same way by running them in a single gel, as a batch. This helped avoiding errors that could arise from laboratory blocking and allowed for parametric tests of significance.

### **3.2.1 Total DNA extraction**

Total DNA was extracted from 0.25 g of finely blended soils by adding 0.5 g of 106 µm diameter glass beads and 1 ml lysis buffer, containing 100 mM Tris-HCl (pH 8.0), 100 mM EDTA, 100 mM NaCl, 1% (wt/vol) polyvinylpyrrolidone and 2% (wt/vol) sodium dodecyl sulfate (SDS) in 2 ml micro-tubes. Samples were homogenized for 30 seconds at 2,500 rpm in a mini-bead cell disruptor (Biospec products Inc.). The samples were centrifuged at 14,000 x g for 1 min at 4° C. A volume of 1/10 of 5 M sodium acetate was added to the supernatant and incubated for 10 min on ice, then centrifuged at 14,000 x g for 5 min. The supernatant was pipetted into sterile 1.5 ml tubes and, 1 volume of ice-cold isopropanol added and placed at -20°C overnight for DNA precipitation. The DNA was pelleted by centrifuging at 14,000 x g, at 4°C for 10 min. The supernatant was pipetted and the pellets washed with 100 µl of 70% ethanol by centrifuging at 14,000 x g for 30 seconds at 4° C. The ethanol was pipetted out and 50 µl of SddH<sub>2</sub>O added. The DNA was run on a 1.0 % agarose gel (stained with ethidium bromide), at 100 V for one hour with a modified protocol (Martin-Laurent et al. 2001). The gel was photographed and visualized under UV illumination using a Bio-Rad gel documentation system.

### **3.2.2 Polymerase chain reaction (PCR) amplification of bacteria**

PCR amplification of bacteria 16S rDNA was based on two primers: gc338f 5' with base sequence ACT CCT ACG GGA GGC AGC AG-3' and 518r 5' with base sequence ATT ACC GCG GCT GCT GG-3' (Muyzer et al. 1993). PCR was carried out in 25 µl reaction volumes containing 2 µl of pure total DNA extract, one dried bead (Ready-to-Go PCR beads, Pharmacia Biotech) and 1.0 µM of each primer, topped to 25 µl with sterile water. For each sample, two PCRs were performed and then pooled together to avoid PCR bias. PCR amplification was performed in a Primus 96 <sup>plus</sup> PCR (MWG AG BIOTECH) touchdown thermal cycler. PCR process involved initial denaturation at 94°C for 2 min, followed by a temperature touchdown program, which consisted of

incubation at 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 1 min for 20 cycles. The annealing temperature (65°C) was lowered in 0.5°C steps within each cycle until 55°C was reached. Ten more cycles were performed, which consisted of incubation at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 min. Finally, incubation was finished with 72°C for 15 min.

### **3.2.3 Polymerase chain reaction (PCR) amplification of fungi 28S rDNA gene**

PCR amplifications were performed using a forward primer 662f with a GC clamp and reverse primer 314r as described by Sigler and Turco (2002). PCR amplifications were carried out in 25 µl reaction volumes, as in bacteria, but reducing the quantity of template DNA from 2.0 µl to 1.0 µl. Cycling conditions were as follows: initial denaturation at 95°C for 10 min followed by 49 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min followed by a final extension phase at 72°C for 10 min. The PCR product (approximately 348 base pairs) was checked for size on a 1% agarose gel stained with ethidium bromide.

### **3.2.4 Denaturing gradient gel electrophoresis**

Denaturing gradient gel electrophoresis (DGGE) for each replicate was done using a DCode universal mutation system (Bio-Rad). The PCR amplification (25 µl) products were loaded onto 8% (w/v) bis/acrylamide gels, which were prepared with a linear gradient ranging from 40 to 60% denaturant (100% denaturant is defined as 7 M urea and 40% (v/v) formamide). Gels were electrophoresed in 1 x Tris-Acetate-EDTA buffer (0.2w/v Tris base; 0.06v/v glacial acetic acid; 0.2v/v EDTA 0.5M pH 8.0) for 16 hours at 60°C and 75 V. After electrophoresis, the gels were stained for 30 min in 100 ml of SddH<sub>2</sub>O containing 7 µl of ethidium bromide and destained in 200 ml SddH<sub>2</sub>O. The gels were photographed and visualized under UV illumination using a Bio-Rad gel documentation system (Muyzer et al. 1993).

### **3.2.5 Data analysis**

DGGE images were captured using Quantity One software ([www.bio-rad.com](http://www.bio-rad.com)) and analyzed using TotalLab software ([www.nonlinear.com](http://www.nonlinear.com)). Construction of dendograms using binary data, based on scores of bands (presence/absence) in fingerprint profiles,

was performed in SPSS software, with the Ward method using squared Euclidean distances. Also, the binary data were used to generate pairwise Jaccard similarity (distance measures) matrices that were then classified using cluster analysis. Cluster analysis was done in SPSS using average linkage between groups. In addition, principal component analysis (PCA) was done by aggregating all the three replicate gels data, and treating each band as a discrete variable. The analysis, with Kaiser Normalization in SPSS, was based on observed band intensity (data logarithm transformed), with absent bands treated as zero intensity.

Diversity of bacteria and fungi was determined using three common biological diversity indices: Richness (S), Simpson's diversity index (D) and Shannon diversity index (H') (Magurran 2004). Richness in this case is the number of rDNA bands observed in DGGE profiles, and such individual band can represent a single species or a taxonomic group of related species and is generally considered as a discrete population (Fromin et al. 2002). Simpson's index, indicating the probability that two individuals drawn from the community belong to the same population, was calculated and its complement obtained, using the formula:

$$D = 1 - \sum_{i=1}^n P_i^2 \quad (3.1)$$

where  $P_i$  is the proportion of intensity of a specific DGGE profile band.

Shannon index was calculated as:

$$H = - \sum_{i=1}^n P_i \ln P_i \quad (3.2)$$

where  $P_i$  is the proportion of an individual band intensity relative to the total intensity of all bands in a PCR-DGGE lane.

Usually, a higher Shannon index is achieved in systems where the number of species (n), identified as discrete bands in gels for our case, is higher and there is evenness among the present species. The diversity indices were subjected to analysis of variance in SAS using the "Mixed" procedure (Federer and King 2007). Separation of least square means was also done using SAS.

### 3.3 Results

#### 3.3.1 Effects on composition and diversity of bacteria communities

##### Composition

Tillage affected bacteria composition in the different sites as revealed by principal component analysis (PCA) of DGGE band intensities (Figure 3.1). In Nyabeda, for example, bacteria communities of all treatments within each of the tillage systems were related ( $P < 0.05$ ), but there were no significant correlations among those across the tillage systems. In addition, PCA for Matayos resulted in greater distances between bacteria communities for treatments with and without crop residue under reduced tillage, than under conventional tillage. In this site, bacteria communities in all the treatments under conventional tillage were related ( $P < 0.05$ ), but under reduced tillage, the communities in treatments without crop residue (except T1) were not significantly related with those in treatments applied with crop residue. Cluster analysis based on presence/absence of bands for each of the sites also revealed similar effects as the PCA (data not shown).

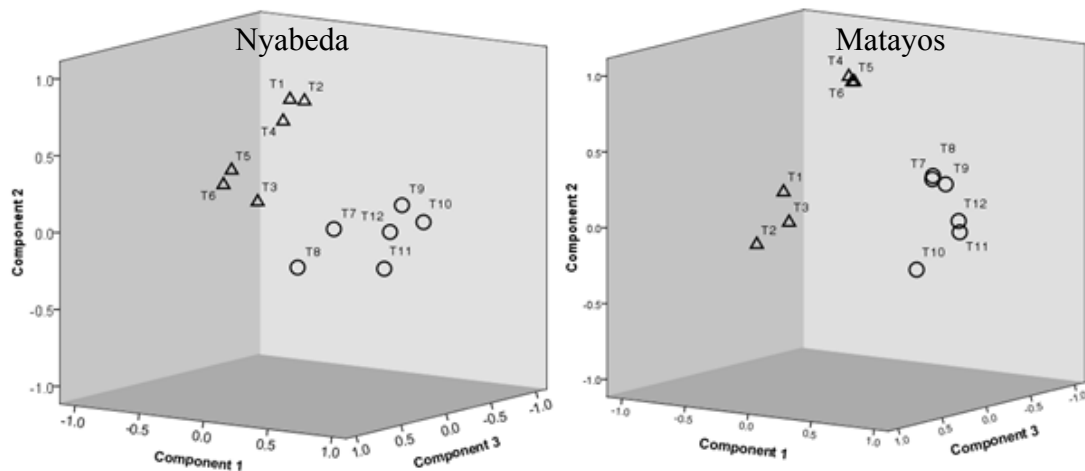


Figure 3.1. Effect of soil fertility management on soil bacteria communities in two sub-humid sites in western Kenya ( $\Delta$  reduced tillage;  $\circ$  conventional tillage); T1-T12 are treatments assessed (see Table 1.2). The three components explained 67% of the total variability in bacteria communities in Nyabeda, and 80% in Matayos

As also observed in the sub-humid zone (Nyabeda and Matayos), tillage had the greatest effect on soil bacteria communities in the dryland site Machang'a (Figure 2). Here, conventional tillage treatments, including the control (also conventionally

tilled), were in one cluster, while those under reduced tillage (tied-ridges and no-till) were grouped into a separate cluster.

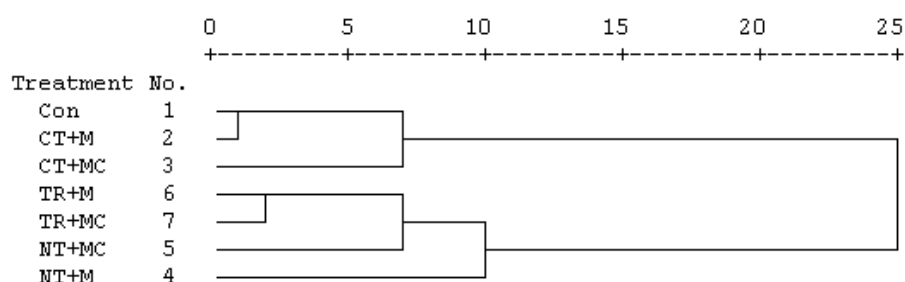


Figure 3.2. Hierarchical clustering of bacteria communities by tillage and organic treatments as observed in Machang'a, eastern Kenya; CT= conventional tillage; TR= tied-ridges; NT= no-till; M= 2 t ha<sup>-1</sup> manure; MC= 1 t ha<sup>-1</sup> manure plus 1 t ha<sup>-1</sup> crop residue (maize stover); Con= control (no organic resource applied)

### Diversity

Diversity indices commonly used in biological studies were also used and revealed additional information not observed with PCA. Cropping system affected diversity of bacteria communities ( $P < 0.05$ ; richness and Shannon indices in Nyabeda); richness, for example, was 25% greater in intercropping and 20% greater in rotation (both having legume) than in the continuous maize system ( $P < 0.05$ ). At the same time, the Shannon index in intercropping was 8% greater than in rotation and 13% greater ( $P < 0.05$ ) than in continuous maize, in treatments without crop residue. The greater diversity in the intercropping system can be attributed to the continuous supply of organic resources of different quality as opposed to the monocrop systems. Cropping system interaction with crop residue affected bacteria diversity ( $P < 0.05$ ; Simpson's and Shannon indices in Matayos; Table 3.1). Generally, crop residue application in Matayos substantially increased bacterial diversity in rotation (by 8% to 9%;  $P < 0.05$ ), decreased diversity in continuous maize (5% to 9%; not significant), while no effect was seen in the intercropping system. This is not surprising since crop residue supplemented high-quality legume biomass in the rotation, which could have benefited the micro-organisms here, while in the continuous maize system, such crop residue can lead to immobilization of some of the available soil nutrients. Unlike with PCA, both tillage and crop residue had no significant effect on bacteria diversity indices. However, the Matayos site showed slightly higher bacteria diversity (+8% Simpsons' and +15%

Shannon indices) in treatments under reduced tillage than those under conventional tillage (data not shown).

Table 3.1. Soil bacteria richness, Simpson's and Shannon indices as affected by crop residue and cropping systems in Nyabeda and Matayos, western Kenya, in 2008

<i>Cropping system</i>	<i>CR</i>	<i>Nyabeda</i>			<i>Matayos</i>		
		Richness (S)	Simpson's index (D)	Shannon index (H')	Richness (S)	Simpson's index (D)	Shannon index (H')
Continuous	-	10.7 <sup>ac</sup>	0.84 <sup>ab</sup>	1.93 <sup>a</sup>	11.5 <sup>a</sup>	0.82 <sup>b</sup>	2.02 <sup>b</sup>
Intercrop	-	13.2 <sup>b</sup>	0.86 <sup>b</sup>	2.19 <sup>b</sup>	11.0 <sup>a</sup>	0.81 <sup>b</sup>	1.96 <sup>b</sup>
Rotation	-	13.0 <sup>b</sup>	0.80 <sup>a</sup>	2.02 <sup>ab</sup>	11.0 <sup>a</sup>	0.74 <sup>a</sup>	1.80 <sup>a</sup>
Continuous	+	10.3 <sup>a</sup>	0.81 <sup>ab</sup>	1.90 <sup>a</sup>	10.8 <sup>a</sup>	0.78 <sup>ab</sup>	1.83 <sup>ab</sup>
Intercrop	+	13.0 <sup>b</sup>	0.81 <sup>ab</sup>	2.13 <sup>ab</sup>	10.5 <sup>a</sup>	0.80 <sup>b</sup>	1.94 <sup>ab</sup>
Rotation	+	12.3 <sup>bc</sup>	0.80 <sup>ab</sup>	2.02 <sup>ab</sup>	11.4 <sup>a</sup>	0.80 <sup>b</sup>	1.97 <sup>ab</sup>
<i>SED</i>		<i>0.818</i>	<i>0.027</i>	<i>0.119</i>	<i>0.932</i>	<i>0.018</i>	<i>0.098</i>
<i>Cropping system</i>		***		*			
<i>CR x Cropping system</i>						*	*

Values in the same column followed by the same letter are not significantly different at  $P=0.05$ . S=number of 16S rDNA bands detected; CR= crop residue applied at 2 t ha<sup>-1</sup>, \*significant at  $P<0.05$ , \*\*\*significant at  $P<0.001$ , SED= standard error of the differences in means

### 3.3.2 Effects on composition and diversity of fungi communities

#### Composition

As with soil bacteria, tillage also influenced community composition of fungi (Figure 3.3). Fungal community population compositions in treatments within each tillage system were highly correlated ( $P<0.01$ ), but there were no significant correlations for communities across the tillage systems. Further, pair-wise treatment comparisons using Jaccard similarity measures showed a wider range of similarity ratios in conventional than in reduced tillage (0.61 to 0.81 in reduced tillage and 0.52 to 0.87 in conventionally tilled treatments). Clustering of these measures to four clusters resulted in three of the

clusters for conventional tillage treatments, and only one cluster for reduced tillage treatments (data not shown).

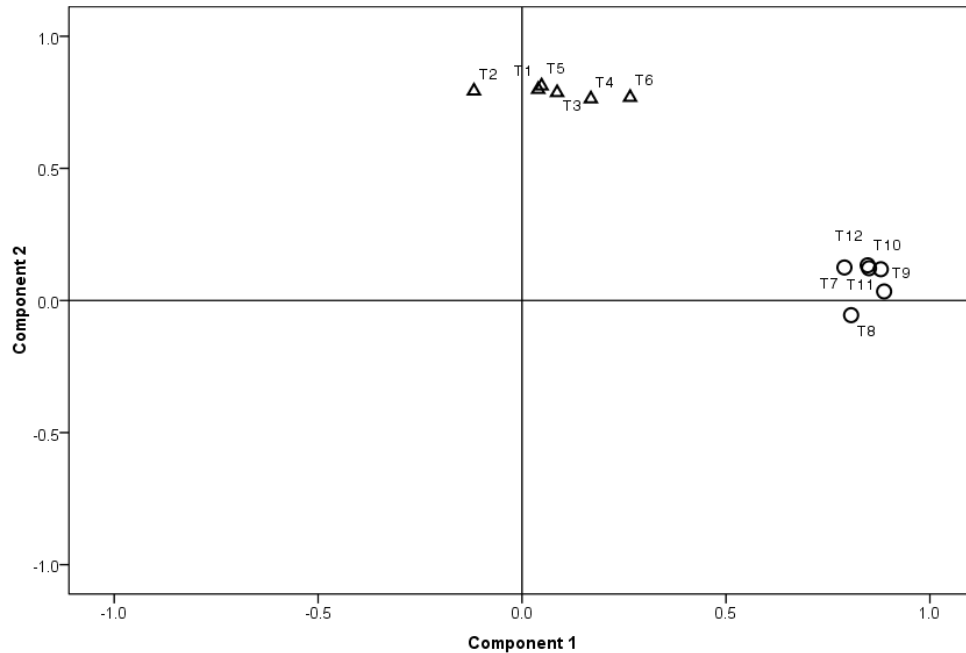


Figure 3.3. Similarity of fungal communities in different treatments in Nyabeda, western Kenya, as defined by principal components of PCR-DGGE profiles ( $\Delta$  reduced tillage;  $\circ$  conventional tillage), T1-T12 are treatments assessed (see Table 1.2). The two principal components accounted for 68% of the total variability of fungi communities

### Diversity

Cropping system affected fungal diversity as observed with the three common diversity indices ( $P < 0.05$ ; Table 3.2). With crop residue application, for example, fungi Simpson's diversity was 24% and 27% higher ( $P < 0.05$ ), and Shannon diversity was 32% and 46% higher ( $P < 0.05$ ) in intercropping than in rotation and continuous maize, respectively. At both crop residue rates, continuous maize had the lowest diversity indices (less by 5% to 32%; except for richness in plots applied with crop residue) compared to cropping systems involving a legume (intercropping and rotation).

Addition of crop residue resulted in lower Simpson's ( $P < 0.05$ ) and Shannon ( $P < 0.01$ ) indices than in treatments without crop residue; the decrease was significant for continuous maize and rotation systems (between -18% and -26% in both cropping

systems;  $P < 0.05$ ) but not for intercropping (-9% to +3%). As with soil bacteria, tillage had no significant effect on soil fungal diversity.

Table 3.2. Soil fungi richness, Simpson's and Shannon indices as affected by cropping system and crop residue in Nyabeda, western Kenya

<i>Cropping system</i>	<i>CR</i>	<i>Richness</i>	<i>Simpson's index</i>	<i>Shannon index</i>
Continuous	-	8.3 <sup>a</sup>	0.72 <sup>cb</sup>	1.55 <sup>ab</sup>
Intercrop	-	10.3 <sup>ab</sup>	0.76 <sup>c</sup>	1.96 <sup>c</sup>
Rotation	-	9.0 <sup>ab</sup>	0.77 <sup>c</sup>	1.82 <sup>c</sup>
Continuous	+	8.8 <sup>a</sup>	0.53 <sup>a</sup>	1.23 <sup>a</sup>
Intercrop	+	12.0 <sup>b</sup>	0.78 <sup>c</sup>	1.79 <sup>bc</sup>
Rotation	+	8.3 <sup>a</sup>	0.63 <sup>b</sup>	1.36 <sup>a</sup>
<i>SED</i>		<i>1.47</i>	<i>0.043</i>	<i>0.117</i>
<i>Cropping system</i>		*	***	***
<i>Crop residue</i>			*	**
<i>Crop residue x cropping system</i>			**	

CR= crop residue applied at 2 t ha<sup>-1</sup>. Values in the same column followed by the same letter are not significantly different at  $P = 0.05$ . \*significant at  $P < 0.05$ ; \*\*significant at  $P < 0.01$ ; \*\*\*significant at  $P < 0.001$ , SED= standard error of the differences in means

A principal component analysis using combined bacteria and fungi DGGE profile datasets was also done. Besides also showing that composition of microbial communities differed between conventional and reduced tillage systems, it revealed that only the communities in continuous maize treatments under reduced tillage (T3 and T6) related positively with those in conventional tillage treatments ( $P < 0.05$ ; Figure 3.4). Due to accelerated residue decomposition following tillage, conventionally tilled systems are likely to be similarly limited in high-quality organic resources as 'continuous maize reduced tillage' treatments, but this is not the case for 'legume based reduced tillage' treatments.



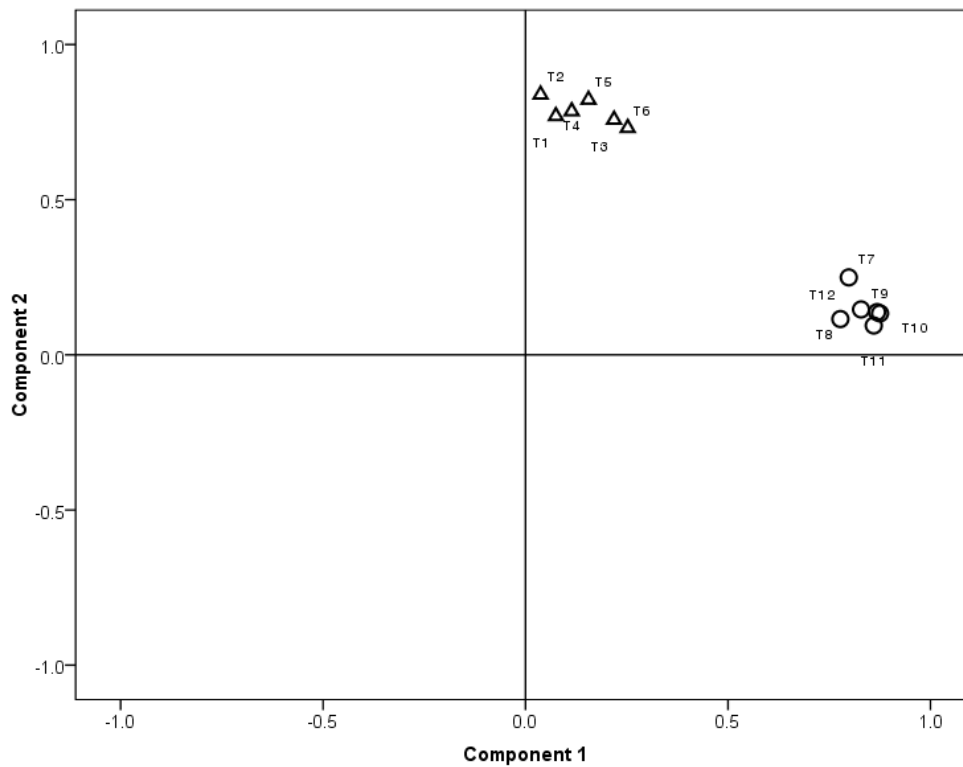


Figure 3.4. Similarity of soil microbial communities (combined bacteria and fungi) in Nyabeda, western Kenya, as defined by principal components of PCR-DGGE profiles ( $\Delta$  reduced tillage;  $O$  conventional tillage), T1-T12 are treatments assessed (see Table 1.2). The two principal components explained 66% of the total variance

### 3.3.3 Microbial diversity and soil chemical properties

Bacterial diversity indices were significantly and positively influenced by soil organic carbon ( $P < 0.05$ ; Figure 3.5), magnesium ( $R^2_{adj} = 0.32$ ,  $P < 0.01$  in Matayos) and total nitrogen ( $R^2_{adj} = 0.16$ ,  $P < 0.05$  also in Matayos; data not shown). The sites where SOC and magnesium related more strongly had the lowest concentrations of these nutrients. Surprisingly, available soil phosphorus related inversely and significantly with bacteria diversity ( $R^2_{adj} = -0.38$  and  $-0.44$ ,  $P < 0.01$ ) in sites which had lowest P. In Nyabeda, where both fungi and bacteria diversity were assessed, a significant positive relationship between bacteria and fungi Shannon diversity ( $R^2_{adj} = 0.15$ ,  $P < 0.01$ ; data not shown) was observed.

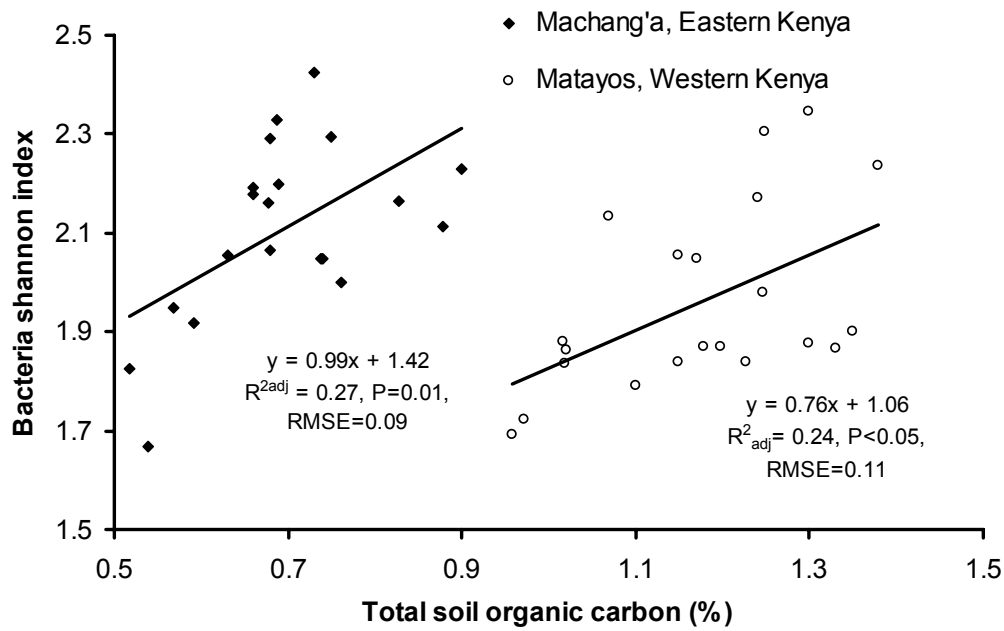


Figure 3.5. Relationship between total soil organic carbon and bacteria Shannon indices in Machang'a (eastern Kenya) and Matayos (western Kenya)

### 3.3.4 Microbial diversity and soil aggregation

The relationship between microbial diversity and soil aggregation indices sampled 5 months earlier (previous season) was established. Since the site had been under similar management for 5 years, and no change of management had taken place prior to microbial sampling, aggregation indices were not expected to change over the short period. In the rotation systems, bacteria diversity related negatively, though not significantly, with aggregation indices. Excluding the rotation system, soil bacteria diversity related inversely with silt-clay content ( $y = -3.15x + 14.7$ ,  $P < 0.05$ ).

Fungi Simpson's diversity indices  $< 0.7$  related inversely ( $P < 0.01$ ) with soil hot water-extractable carbon and with aggregate mean weight diameter (Figure 3.6). However, fungi Simpsons' indices  $> 0.7$  related positively (not significant) with soil aggregate mean weight diameter and with hot water-extractable carbon (data not shown).

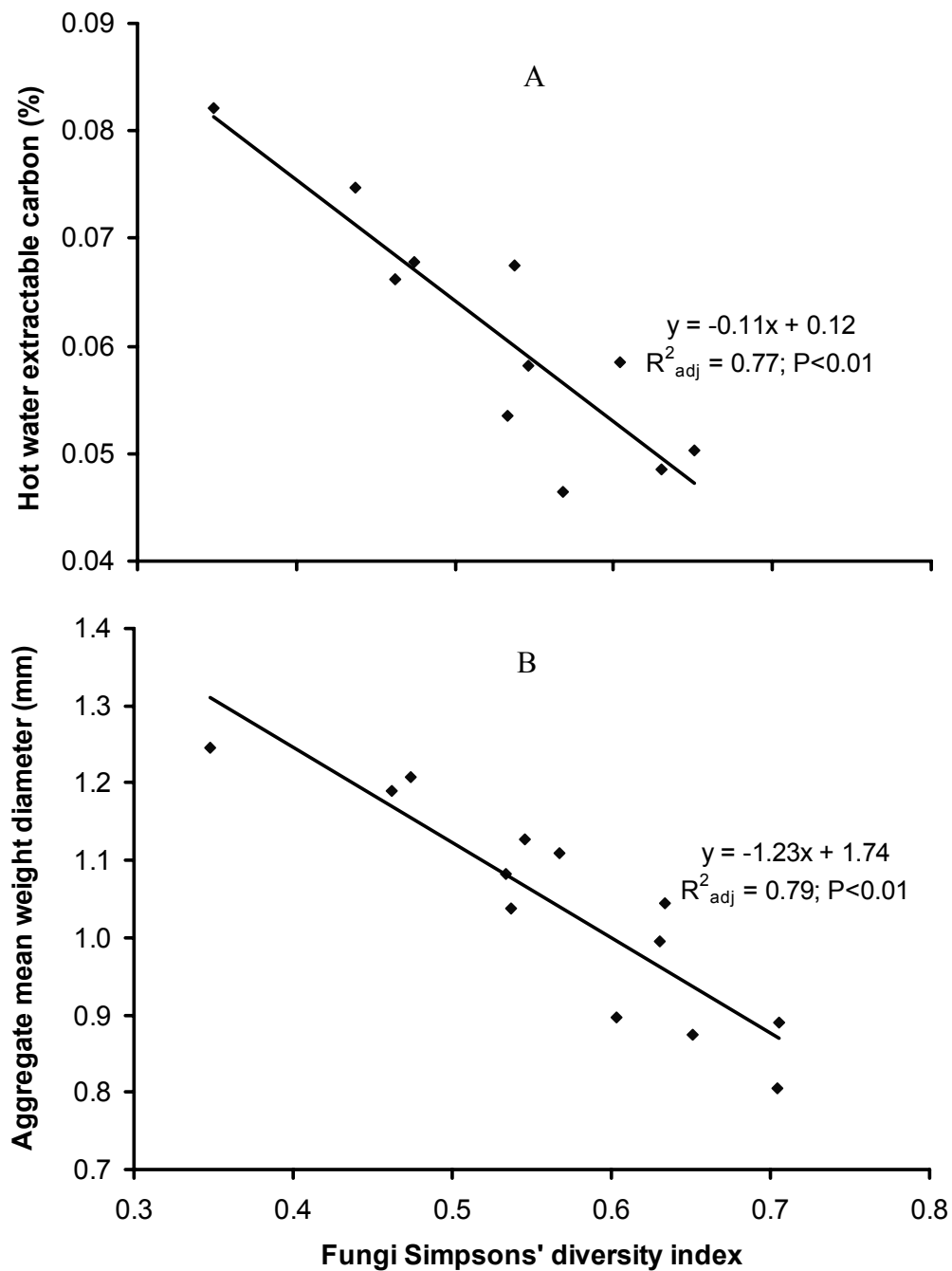


Figure 3.6. Relationship between Simpson's diversity indices of soil fungi and hot water-extractable carbon (A) and aggregate mean weight diameter (B) as observed in Nyabeda, western Kenya

### **3.4 Discussion**

#### **3.4.1 Composition and diversity of bacteria**

Tillage (soil disturbance) has been identified as one of the important factors affecting microbial communities, along with moisture and residue placement (Six et al. 2006). Band-matching techniques revealed contrasting bacteria community composition between reduced and conventional tillage, i.e., the differences indicate shifts in the composition of bacteria community due to tillage. Similar findings of tillage effect on bacteria community composition were reported in Brazil, in a similar soil type as in Nyabeda (Peixoto et al. 2006). However, only a slight tillage effect was indicated using common biological diversity indices. While diversity indices (e.g., Shannon and Simpson's indices) capture only quantitative changes, band analysis is expected to also capture qualitative changes, e.g., replacement of one guild of bacteria with another. This would have no effect on total richness, but the presence or absence of certain species would result in differences in species assemblage composition captured by band-matching techniques (Nakatsu et al. 2005). The slightly lower bacteria diversity in the disturbed system (conventional tillage) is in agreement with the reports of other researchers (Øvreås and Torsvik 1998; Six et al. 2006).

Crop residue has an important role in defining bacteria community composition and diversity in soil through its role as a carbon source (Gelsomino and Cacco 2006; Nakatsu et al. 2005). The greater distances between different residue treatments in reduced tillage observed with the cluster analysis indicate that crop residue application could have a stronger influence on the bacterial community composition under reduced tillage than under conventional tillage. Concentrating crop residue on the soil surface, as in reduced tillage, has additional microclimate effects such as soil moisture conservation and regulation of soil temperature; this is consistent with a greater response of bacteria community composition to surface-placed than to incorporated crop residue in our study. A similar result was observed in a clay soil under long-term residue management in southern Italy, where incorporated crop residue had no effect on soil microbial communities (Crecchio et al. 2006). But abundance of particular bacteria species has also been shown to vary (some bacteria species increase while others decrease abundance) depending on the rates of carbon mineralization

(Fierer et al. 2007), and the mineralization rates are usually different for incorporated and surface-placed residue besides also varying with time.

Our results on combination of manure and crop residue imply that such combination could provide a greater compositional shift in bacteria communities as opposed to application of manure alone. However, literature on effects of such combinations on soil bacteria communities is lacking and calls for further investigations in order to make conclusions. Also, although band analysis provides an improvement over diversity analysis alone, it still is not sufficient to provide insight into the physiological preferences of the bacteria species available, and therefore provides only limited information.

Cropping systems affect microbial diversity (and community composition) through variability in composition and structure of organic matter, which serves as microbial nutrient and energy source (Agnelli et al. 2004). The relative abundance of different bacteria species may depend on plant type (Smalla et al. 2001), as bacteria usually respond differently to different compositions of crop plant exudates (Garbeva et al. 2004), based on their copiotrophic or oligotrophic attributes that correspond to *r*- and *K*-selected categories (Fierer et al. 2007). Higher diversity of bacteria in the intercropping system in both sub-humid sites could be explained by the wider range of organic substrates (Øvreås and Torsvik 1998), contrary to monocropping systems. Intercropping system is characterized by a continuous supply of a mixture of low- and high-quality substrates and a modified microclimate, which could favor co-existence of a wide range of microbial species. This contrasts with continuous maize where low-quality organic resources predominate, with possible nutrient immobilization and a limitation of microbial species (Alden et al. 2001). It also contrasts with the maize-soybean rotation system where low- and high-quality organic resources become successively available and where a corresponding succession of microbial species is expected. Seasonal changes from monocrop legume to monocrop cereal as the carbon source in rotation, without some cereal residues during the legume phase, could contribute to the lower diversity of this system. Indeed, the significant response of bacteria to crop residue application (Simpsons' index in Matayos) in the rotation system suggests, perhaps, that some of previous crops' residues should be left on the plots to sustain diversity of micro-organisms in this cropping system. Therefore, increasing

carbon while also supplementing with higher quality biomass, as in the intercropping system, could help to avoid nutrient limitations, and achieve increased diversity of soil microbes. However, further research would be needed to separate these effects from each other.

### **3.4.2 Composition and diversity of fungi**

As with soil bacteria, similarities of soil fungal community composition within and not across tillage practices indicate a compositional shift in fungal communities among the tillage systems. Similar effects of tillage on fungal community structure are reported for temperate soils (Jansa et al. 2002) and for tropical soils (Cookson et al. 2008). The soil perturbation that is associated with tillage has been associated with changes in substrate (organic carbon) availability and utilization (Cookson et al. 2008) that result in differences in fungi responses. Also, the perturbation result in instability of microbial communities (Øvreås and Torsvik 1998), as was also observed in our site, with more dissimilar fungal communities in the conventional compared to the reduced tillage system.

Besides affecting shifts in microbial community composition, reduced tillage systems have greater fungal growth (Beare et al. 1993; Degens et al. 1996). Thick dark bands in the DGGE profiles for the reduced tillage treatments in Nyabeda are consistent with higher fungal abundance when compared to conventional tillage (data not shown).

Reduced fungal diversity following addition of crop residue implies that the application of maize stover, which usually is of low quality, favored a few populations of fungi that could utilize such residue, as also reported by Marschner et al. (2003). The case of reduced diversity of fungi by low-quality organic resources is exemplified by the lowered indices in continuous maize cropping system compared to the legume-based systems in almost all cases. Consistent with the results observed in the present study, higher fungal diversity in cropping systems involving rotations (and intercropping), as opposed to continuous systems, has been reported elsewhere (Bremer et al. 2007; Broeckling et al. 2008). Although soybean-maize intercropping had highest fungi diversity (similarly bacteria), this cropping system has received little attention in microbial diversity studies relative to sole cropping systems.

An integrated analysis of both bacteria and fungi datasets in characterization of soil fertility management options can result in revelation of additional information, thus giving an even more realistic picture. Yet, to our knowledge, no such combined analysis of fungi and bacteria PCR-DGGE profiles has been undertaken in characterization of agricultural soils. The similarity of only continuous maize (and not legume-based) treatments under reduced tillage with conventional tillage treatments that was revealed in the combined analysis could indicate that low-quality maize-derived carbon predominates in the carbon pool in conventional tillage. This makes sense, since high-quality legume residues can decompose more rapidly under conventional tillage, thus the contribution to soil micro-organisms is small compared to under reduced tillage. The remaining high C:N residues could then characterize the system's microbial community. Our study provides an indicator of the variability of microbial community composition among different treatments. However, since microbial community composition changes between seasons (Ping et al. 2009; Smit et al. 2001), and such changes are expected especially where crops are rotated, analysis over several seasons is suggested.

Since rare species are not taken into account, it is not clear how they would influence microbial diversity indices. While species-abundance measures based on counts of organisms in ecological studies take into account species even with only one individual (Magurran 2004), the microbial measures based on DGGE profiles take into account species with a proportional abundance above a certain minimum threshold (Hedrick et al. 2000), i.e., usually 1% of the total target DNA. Microbial diversity measures, as in our case, therefore, represent diversity of the most abundant microbial assemblages.

### **3.4.3 Microbial diversity and soil chemical properties**

Microbial diversity related positively with soil chemical properties, as expected. Positive relationship of bacterial diversity with soil nutrients such as organic carbon has also been reported elsewhere (Marschner et al. 2003). In our case, the response was dependent on the degree of nutrient limitation, being higher in the more limiting sites. For example, significant responses of bacteria diversity to soil organic carbon and magnesium were found in the sites where the nutrients were most limiting. The inverse

and significant response of bacteria diversity to available P, only in sites of lowest P (Nyabeda and Machang'a), was surprising. It is likely that, under P limitation, an initial increase in P resulted in higher predominance of the microbial community structure by fewer advantaged species, and above a threshold of available P further increases benefited all bacterial species. Thus, farming practices that improve availability of soil nutrients also enhance soil microbial diversity, but some initial decline in diversity can result in some cases as found with soil P.

Despite sandy texture and low carbon content in the dryland site, bacteria diversity was higher than in the sub-humid sites, perhaps due to the almost neutral pH in the dryland site. In a continental-scale research involving different sites in North and South America, diversity of soil bacteria communities increased as soil pH increased from acidic to near neutral (Fierer and Jackson 2006).

#### **3.4.4 Microbial diversity and soil aggregation**

The relationship between soil bacteria diversity with silt-clay content indicates that bacteria diversity contributed to processes aggregating or stabilizing fine soil particles. The significant relationship with only silt and clay could be due to the fact that bacteria mucilage affect mainly the formation of microaggregates (Six et al. 2004) from silt and clay, as well as the stabilization of the formed aggregates (Alami et al. 2000; Amellal et al. 1998).

Fungi affect soil structure through enmeshment of soil particles by fungal hyphae and stabilization of these aggregates by their extracellular polysaccharides (Six et al. 2004; Tisdall 1994). Our data suggest that for diversity <0.7, an increase in fungi community diversity resulted in a decrease in soil macroaggregation. Such decrease may be due to differences in the effectiveness of different fungi species (present at different diversity levels) to stabilize aggregates (Tisdall 1994). Thus, fungus species effective in soil macroaggregation thriving at low diversity could be replaced by less effective fungi species as diversity increased. After attaining some level of diversity (at least 0.7 in this case), further increase in diversity increased macroaggregation, which could be related to a better stabilization effect of extracellular polysaccharides from varied fungi species.



In summary, for each of the three sites, tillage was the most important factor determining composition of microbial communities, dominating the effects of crop residue and cropping systems. While tillage, and to some extent crop residue, had a strong effect on the composition of microbial communities, cropping system mainly affected relative abundances of existing microbial species. Since band-matching techniques and diversity indices revealed different microbial information, the use of different techniques to extract information from DGGE profiles is suggested for future studies. Also, future work should investigate which of the microbes are enhanced and which are suppressed by different land-use practices in order to make microbial findings more useful for management.

### **3.5 Conclusions**

Reduced tillage affected microbial composition as revealed by band-matching and PCA technique, but had only a slight increase on the microbial diversity indices used in biological studies, relative to conventional tillage. Application of crop residue resulted in compositional shifts in bacteria communities in one site (Matayos), and resulted in 18% to 26% lower fungi diversity (Shannon and Simpson's indices in continuous maize and rotation systems) than in treatments without crop residue. With crop residue application, practicing soybean/maize intercropping resulted in 24-46% and 0-12% more fungi and bacteria diversity, respectively, than in systems involving monocropping, which supports our second hypothesis. Soil microbial diversity related inversely and positively with soil aggregation for diversity indices  $<0.7$  and  $>0.7$ , respectively. Thus, our first and third hypotheses were only partially supported. Future microbial diversity studies should give more attention to soybean/maize intercropping than presently done. Combination of reduced tillage and simultaneous supply of low- and high-quality organic resources, as in the intercropping system, is suggested as one of the best strategies to promote diversity of soil bacteria and fungi.

## **4 CROP RESIDUE DISAPPEARANCE AND MACRO- AND MESOFAUNA ACTIVITY**

### **4.1 Introduction**

Surface crop residue management is an important requirement in conservation tillage, and at least 30% surface cover at planting is recommended (Fowler and Rockstrom 2001). Surface crop residue protects soil from erosion and surface evaporation, and adds nutrients to the soil when mineralized (Wang et al. 2006). Attaining sufficient surface cover by crop residue is one of the main challenges in conservation tillage in sub-humid tropical environments in Africa. In most cases, availability of crop residues for use as mulch in smallholder farming systems is limited by low productivity, and by the competing uses of these resources, such as for fuel wood and fodder (Erenstein 2003). Village-level residue management study in West African agro-ecosystems showed that 21% to 39% of residue from previous crops is available on the farm at planting (Bationo et al. 2007). This indicates that a majority of smallholder farmers can afford to apply about 2 t ha<sup>-1</sup> of residue from the previous crops on farm fields especially in the sub-humid zones where biomass production is usually high. But such crop residue applied or left on the farm after harvest has been found to disappear quickly (Ouédraogo et al. 2004), mainly depending on climate, soil type, residue quality, placement and soil fauna activities (Ouédraogo et al. 2004; Pouyat and Carreiro 2003; Vesterdal 1999; Wang et al. 2006). The fast disappearance of crop residue could expose the soil and reduce the envisaged benefits. In Kenya, the rate of on-farm disappearance of maize stover applied as crop residue in reduced tillage systems had never been assessed before.

Soil fauna plays an important role in primary decomposition, the mechanical breakdown of organic residues, and is key to improvement of soil structure and distribution of soil nutrients (Brown and Whitford 2003; Kurzatkowski et al. 2004). Soil macro- and mesofauna, especially termites, are the greatest challenge to applied crop residue in western Kenya, as is the case in other tropical environments (Fowler and Rockstrom 2001; Mando and Brussard 1999). The high C:N ratio of, for example, the maize stover often used as crop residue, should result in its slow decomposition (Tian et al. 1995), and hence longer persistence on the soil surface, but macro- and mesofauna comminute such residues and this results in their fast depletion. The activity of macro-

and mesofauna is affected by microclimate status such as temperature variation and drought stress (Martius et al. 2004). As tillage and cropping systems usually influence soil microclimate, this study was undertaken assessing residue disappearance in different tillage practices and the cropping systems commonly used by farmers.

A major contribution of termites is re-organization and molding of large volumes of soil along their feeding trails, as they usually cover surface residues, fallen leaves and maize stems (Fernandes et al. 1997; Nhamo 2007) with thin crusts of organic material (often called “galleries”, but we use the term “surface sheeting” instead). Termites also mold soil into mounds, which are very predominant in African landscapes and cover a substantial proportion of the soil area (Fall et al. 2001). The termite surface gallery/sheeting and mound soil is usually mixed with organic glues and has higher carbon content than the surrounding farm soil (Brauman 2000). Two genera of termites, *Pseudacanthotermes* and *Synacanthotermes* of the fungus-growing Macrotermitinae subfamily of the family Termitidae (Krishna 1970) are active at the experimental site in western Kenya (Ndabamenye 2006). They mold soil into temporary structures that disintegrate after a moderate or high rainfall event. Although some studies have determined particle sizes and densities of termite soil (Amelung et al. 2002; Nhamo 2007), no study could be found assessing aggregate stability of on-farm termite-molded gallery soils.

The aim of this study was to (i) characterize crop residue disappearance during crop growth, and (ii) assess termite activity and characteristics of termite-molded gallery soil (carbon concentration and aggregate stability) in a conservation tillage experiment conducted in Nyabeda, western Kenya. The hypotheses tested were that:

- (i) Macro- and mesofauna contribute more to disappearance of added crop residue than other biotic and abiotic factors, and
- (ii) On-farm termite-molded surface sheeting soils are less water-stable, and are more enriched in carbon than the bulk farm soil.

## **4.2 Materials and methods**

### **4.2.1 Crop residue disappearance**

This study was conducted in a conservation tillage experiment established since 2003 in Nyabeda, western Kenya, and managed by the African Network for soil biology and fertility (AfNet) of the Tropical Soil Biology and Fertility (TSBF) institute of CIAT (see section 1.5).

Surface crop residue disappearance was determined in both reduced and conventional tillage systems. Belowground residue assessment was done only in the conventional tillage system to avoid disturbance of reduced tillage treatments in the ongoing trial. Four treatments, replicated 3 times, were used in this assessment, i.e., rotation and continuous maize under both reduced and conventional tillage. All treatments had crop residue added. Eight litterbags each of 5 mm and 1 mm mesh sizes were used per conventional tillage plot. The 5 mm litterbags were made of tough rubber, while the 1 mm litterbags were of lighter plastic material as no tougher material could be found. Half of the litterbags were buried in the plough layer, at about 10 cm depth, while the other half was placed on the soil surface. A similar number of litterbags as on surface of the conventional tillage plots were placed on the surface of the reduced tillage plots. In total, we used 192 litterbags. Crop residue (50 g of maize stover on dry weight basis) was placed in each litterbag at the beginning of March-August 2007 cropping season. The coarse 5 mm litterbags allowed soil macro- and mesofauna, mainly termites, to access crop residue, while the fine 1 mm litterbags restricted the access. For each plot, one of each type of litterbag was retrieved at 28, 49, 77 and 105 days after planting (corresponding to 4, 7, 11 and 15 weeks after planting, respectively). During the last two samplings, some of the 1 mm litterbags had been broken into by termites and were therefore considered as missing in the statistical analysis. Crop residues recovered from the litterbags were carefully washed with water to remove soil, oven-dried at 105°C for 48 hours and resulting dry weights determined. The loss of crop residue from the litterbags is termed here as disappearance. During the subsequent season, crop residue remaining on plot surface was collected on a monthly basis, attached soil removed by brushing it out, and the residue was then ground for determination of chemical composition (lignin, ash content, carbon [C], nitrogen [N], phosphorus [P] and potassium [K]).

The contribution of soil macro- and mesofauna to disappearance of crop residue was calculated using the following formula from Ouédraogo et al. (2004):

$$D = \frac{A - B}{100 - B} \times 100 \quad (4.1)$$

where D is percent residue disappearance due to macro- and mesofauna, A is the percent of organic material remaining in the absence of soil macro- and mesofauna (i.e., in the litterbag of 1 mm mesh size), and B is the percentage of organic material remaining in the presence of soil macro- and mesofauna (i.e., in the litterbag of 5 mm mesh size).

#### **4.2.2 Termite activity**

Termite activity was assessed by counting surface sheetings in plots under soybean-maize rotation (separately for soybean and maize plots), soybean-maize intercropping and continuous maize at 14 and 16 weeks after planting. Surface sheetings are defined here as thin termite crusts on organic resources (applied crop residue and stems of standing maize) and covered runways that are at least 5 cm long. Belowground termite activity was assessed within conventionally tilled sole maize and soybean plots in two 25 cm by 2 m quadrats. The quadrat areas were dug using a hand-hoe, and all incorporated crop residue (maize stover) that had visible termite activity, as well as other termite materials such as termite fungus combs, counted. Termite-molded surface sheeting soil was also collected for the two assessment periods, bulked and analyzed for soil organic carbon and aggregate stability. In addition, soil used in the construction of two termite nests on the farm were sampled and subjected to similar analysis as the surface sheeting soil. The termite nests were constructed during the cropping season of assessment.

#### **4.2.3 Analyses**

Laboratory analyses for plant and soil samples were done following the procedures described previously (see Chapter 1). Determination of aggregate stability of the termite-molded surface sheeting and mound soil was done using a wet-sieving method (see section 2.2).

Analysis of variance and separation of least square means was done using SAS software. For the termite activity, counts were log transformed (ln) before analysis of variance.

### **4.3 Results**

#### **4.3.1 Crop residue disappearance**

Litterbag mesh size affected ( $P < 0.001$ ) crop residue disappearance at all sampling times for both surface-placed and buried crop residue, with greater residue loss in the presence (5 mm mesh size) than in the absence (1 mm mesh size) of macro- and mesofauna (Figure 4.1). For example, there were 1.5 to 8 times and 0.5 to 2.3 times less residue remaining in the litterbags allowing macro- and mesofauna than in those restricting the fauna, for surface-placed and buried residue, respectively. The differences were greater at the beginning and decreased steadily to the final sampling, perhaps due to changes in residue quality. Similarly, litterbag placement affected ( $P < 0.05$ ) residue disappearance for all sampling periods. For the litterbags limiting macro- and mesofauna access to crop residue, 13% to 34% less residue remained in the buried than in the surface-placed litterbags in all sampling periods ( $P < 0.05$ ). Also, for bags that allowed unrestricted access, 23% to 34% less residue was recovered from buried litterbags than from surface-placed litterbags during the first 3 samplings (not significant). The greater accessibility of buried residue to soil fauna  $< 1$  mm (mesofauna) was likely the reason for the greater residue loss in buried than surface-placed residue. On average, we recovered 14% less crop residue from buried litterbags in the rotation than in the continuous maize system (data not shown); perhaps the microclimate under the soybean resulted in increased abundance of soil fauna than under continuous maize. There was no effect of tillage on crop residue disappearance in this study (data not shown).

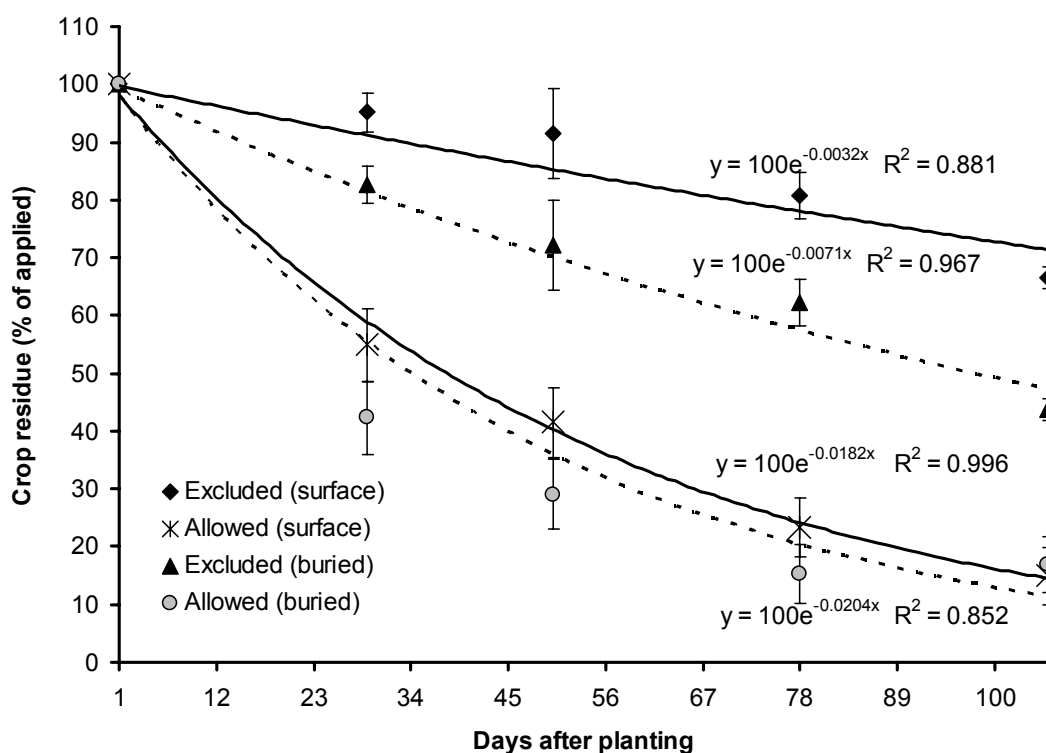


Figure 4.1. Surface and buried crop residue disappearance in western Kenya, with ('allowed') and without ('excluded') macro- and mesofauna attack, in March-August 2007 cropping season. Macro- and mesofauna excluded = 1 mm mesh size litterbags. Macro- and mesofauna allowed = 5 mm mesh size litterbags. Bars are least significant differences (LSD)

The relative contribution of macro- and mesofauna to disappearance of surface-placed residue was higher (by 10% to 50%;  $P < 0.05$ ) than that of buried residue during all sampling times (Figure 4.2), attributed to possibly more easy accessibility of surface-placed residue to a wider range of soil  $>1$  mm macro- and mesofauna than was the buried residue. Thus, although overall residue disappearance was faster for buried than for surface-placed residue due to greater accessibility to  $<1$  mm soil fauna as explained earlier, macro- and mesofauna were more active on the surface. Also, the contribution of macro- and mesofauna to residue disappearance declined with time from about 90% at 28 days after planting to 60% at 105 days after planting for surface-placed, and from 70% to 28% for buried residue for the same periods. This decline was expected due to declining residue quality. The generally larger contribution of macro- and mesofauna to residue loss than other soil organisms can mainly be attributed to the

large number of termites that are active at the site. In contrast, the role of other soil fauna is greatly reduced.

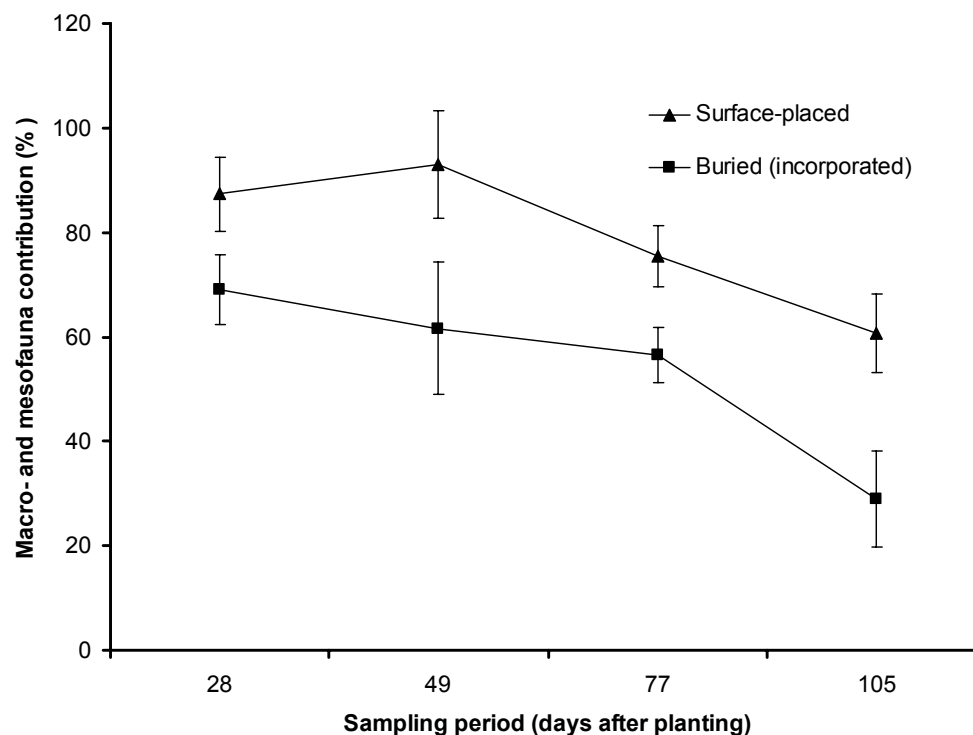


Figure 4.2. Contribution of macro- and mesofauna to surface-placed and incorporated crop residue disappearance in western Kenya, in March-August 2007 cropping season; error bars represent least significant differences

Chemical composition of the surface-placed crop residue, monitored on a monthly basis, showed a slow decrease in C content ( $P < 0.05$ ) as expected, and the carbon was 5% to 19% lower in the subsequent months than in the first month. Compared to the first month, there were substantial increases in N (25% to 56%) and P concentration (25% to 50%;  $P < 0.05$ ) in subsequent months, perhaps resulting from importation of enriched organics in termite saliva. The fast decrease in K content (76% to 84%;  $P < 0.01$ ) with time relative to the first month was expected due to leaching in rainwater (Table 4.1). Polyphenol was not affected by sampling time, although in general there was a 10% to 31% lower polyphenol content in the months after the start. Lignin increased 110% in the first month; the crop residue had 95% to 170% higher ( $P < 0.05$ ) lignin contents for all other months than in the first month. Ash content was 60% higher (not significant) in the second and third months than in the first month, but



increased sharply by 260% during the fourth month ( $P < 0.01$ ). The sharp increase in ash content could not be confirmed as the data is reported for one season only.

Table 4.1. Chemical composition of surface-placed crop residue at monthly intervals after application as observed in western Kenya in September 2007 - January 2008 cropping season

<i>Month</i>	<i>% C</i>	<i>% N</i>	<i>% P</i>	<i>% K</i>	<i>% Polyphenol</i>	<i>% Lignin</i>	<i>% Ash</i>
1	40.36 <sup>b</sup>	0.36 <sup>a</sup>	0.04 <sup>a</sup>	0.98 <sup>b</sup>	0.51 <sup>a</sup>	4.02 <sup>a</sup>	5.33 <sup>a</sup>
2	37.89 <sup>ab</sup>	0.56 <sup>b</sup>	0.05 <sup>ab</sup>	0.24 <sup>a</sup>	0.44 <sup>a</sup>	8.40 <sup>bc</sup>	8.71 <sup>a</sup>
3	38.28 <sup>ab</sup>	0.48 <sup>ab</sup>	0.05 <sup>ab</sup>	0.18 <sup>a</sup>	0.46 <sup>a</sup>	10.84 <sup>c</sup>	8.53 <sup>a</sup>
4	32.79 <sup>a</sup>	0.45 <sup>ab</sup>	0.06 <sup>b</sup>	0.16 <sup>a</sup>	0.35 <sup>a</sup>	7.84 <sup>b</sup>	30.49 <sup>b</sup>
5	35.00 <sup>ab</sup>	0.55 <sup>b</sup>	0.06 <sup>ab</sup>	0.20 <sup>a</sup>	0.41 <sup>a</sup>	8.65 <sup>bc</sup>	24.45 <sup>b</sup>
<i>SE</i>	<i>2.103</i>	<i>0.059</i>	<i>0.007</i>	<i>0.075</i>	<i>0.062</i>	<i>0.810</i>	<i>3.604</i>
<i>Sampling time effect</i>				***		**	**

SE= standard error of means, \*\*\*significant at  $P < 0.001$ , \*\* significant at  $P < 0.01$

#### 4.3.2 Termite activity

Crop type dramatically affected ( $P < 0.05$ ) surface termite activity; rotation plots under soybean showed 50% of the termite activity of rotation plots under maize and of the control plots (maize) without crop residue (Table 4.2; maize with or without residue application showed no difference in termite activity). The presence of ants under soybean (visually observed and attracted by aphids on soybean stems and leaves) could keep termites away, hence the reduced activity under this crop. In the control, the termite activity was located mainly at the base of maize stems, which was not surprising as maize would be an alternative food source in the lack of other resources. There was no difference in belowground termite activity. Also, there was no significant effect of tillage on surface termite activity, although termite activity was higher by 14% under reduced tillage than under conventional tillage (data not shown). One has to consider, however, that these counts generically reflect termite activity without accounting for any species differences, which makes it difficult to extract information from this kind of assessment.

Table 4.2. Effect of crop on surface (termite-molded sheetings) and belowground termite activity in conventional tillage treatments in western Kenya in 2007

<i>Crop</i>	<i>Crop residue</i>	<i>Surface activity</i>	<i>Belowground activity</i>	
			Burrows <sup>#</sup>	Stover attacked
Maize (control)	-	3.2 <sup>b</sup>	nd	nd
Monocropped maize	+	2.8 <sup>ab</sup>	2.2 <sup>a</sup>	0.4 <sup>a</sup>
Rotated maize	+	3.2 <sup>b</sup>	2.0 <sup>a</sup>	0.3 <sup>a</sup>
Rotated soybean	+	1.7 <sup>a</sup>	2.6 <sup>a</sup>	0.6 <sup>a</sup>
Intercropped maize/soybean	+	2.7 <sup>ab</sup>	nd	nd
<i>SE</i>		0.28	0.23	0.28
<i>Crop effect</i>		*		

Crop residue was applied at 2 t ha<sup>-1</sup>; data were averaged for the 14 and 16 weeks after planting sampling periods; <sup>#</sup>represent termite holes and fungus combs; data are log-transformed counts of termite structures and attacked stover, SE= standard error of means; \*\* significant at P<0.05; nd= not determined

Gallery soil, mainly termite sheeting, from the three cropping systems (soybean-maize rotation, soybean/maize intercropping and continuous maize) had 11% to 26% and 25% to 42% higher (P<0.01) carbon than farm soil and termite nest/mound soil, respectively (Table 4.3). This is consistent with the fact that termite-molded surface sheeting soil was thoroughly mixed with termite saliva. Carbon in termite mound soil was similar to that in bulk farm soil, but significantly lower (P<0.05) than in all termite-molded surface sheeting soils tested. The mounds were small, about 30 cm wide and 10 cm high, as they were just being formed and their soil, representing the outer mound wall, had been imported by termites from the subsoil where carbon content is low. Carbon in termite-molded surface sheeting soil was 14% higher (P<0.05) in the intercropping and 11% higher (not significant) in the rotation than in the continuous cereal system, perhaps reflecting differences in carbon of the very top soils in these cropping systems.

Table 4.3. Carbon (%) from termite-molded surface sheeting soil under different cropping systems, as compared to mound and bulk farm soil (0-5 cm depth) in western Kenya, March-August 2007 cropping season

<i>Treatment</i>	<i>Carbon (%)</i>
Continuous	1.52 <sup>bc</sup>
Intercrop	1.73 <sup>a</sup>
Rotation	1.69 <sup>ab</sup>
Mound	1.22 <sup>d</sup>
Bulk farm soil (0-5 cm depth)	1.37 <sup>cd</sup>
<i>SE</i>	0.069

SE= standard error, values followed by the same letter are not significantly different at  $P < 0.05$

#### 4.3.3 Aggregate characteristics of termite-molded gallery and mound soil

Soil aggregate distribution in the farm soil was in the order small macroaggregates (SM) > microaggregates (Mi) > silt+clay (S+C) > large macroaggregates (LM; Table 4.4). Previously, we reported 30-89% higher aggregation in reduced than in conventional tillage (Chapter two). The size distribution in termite-molded surface sheeting and mound soils followed the same general pattern as in the farm soil, with the largest proportion (43% to 67%) being in small macroaggregate and the smallest (0.1% to 7%) in large macroaggregate fractions. Except for the ‘conventional tillage + continuous maize’ treatment, which had 78% less large macroaggregates, 24% more microaggregates and 30% more silt+clay ( $P < 0.05$ ), we found similar aggregate sizes in termite-molded surface sheeting soil relative to the farm soil. It may be that for the ‘conventional tillage + continuous maize’ treatment, a large proportion of the wet-sieved soil consisted of aggregates brought up from the subsoil by termites. In contrast to termite-molded surface sheeting soils, termite nest (mound) soil had elevated small macroaggregate (by 22% to 56%;  $P < 0.05$ ) and lowered silt+clay (by 47% to 98%;  $P < 0.05$ ) soil fractions compared to all treatments tested. Generally, reduced tillage had

3% to 78% higher large aggregate fractions and 11% to 20% higher small macroaggregates and similarly lowered microaggregates compared to conventional tillage, although these differences were mainly not significant. This was interesting, as the same trend had been reported for bulk farm soil, and a tendency for increased aggregate sizes in intercropping relative to continuous maize and rotation was similar to our previous report for on-farm aggregation. Gallery/sheeting material had 3 to 12 times more particulate organic matter (POM) than farm soil and mound; as already said, it is likely that some organic debris were glued to the aggregate surfaces by termite saliva during molding of surface sheetings.

Table 4.4. Aggregate sizes of termite-molded surface sheeting and mound soil as observed in Nyabeda, western Kenya, March-August 2007 cropping season

<i>Treatment</i>	<i>Aggregate fraction ---g 100g<sup>-1</sup> soil---</i>				
	LM	SM	Mi	S+C	POM
Conventional tillage + continuous	1.2 <sup>b</sup>	43.2 <sup>d</sup>	44.5 <sup>a</sup>	11.5 <sup>a</sup>	0.10 <sup>bc</sup>
Reduced tillage + continuous	5.4 <sup>a</sup>	47.8 <sup>bcd</sup>	36.6 <sup>abc</sup>	10.4 <sup>ab</sup>	0.24 <sup>ab</sup>
Conventional tillage + intercrop	6.5 <sup>a</sup>	49.9 <sup>bcd</sup>	35.1 <sup>bc</sup>	9.5 <sup>ab</sup>	0.15 <sup>bc</sup>
Reduced tillage + intercrop	6.7 <sup>a</sup>	55.5 <sup>b</sup>	29.6 <sup>c</sup>	8.5 <sup>b</sup>	0.33 <sup>a</sup>
Conventional tillage + rotation	3.2 <sup>ab</sup>	43.5 <sup>cd</sup>	43.4 <sup>ab</sup>	10.4 <sup>ab</sup>	0.17 <sup>bc</sup>
Reduced tillage + rotation	5.7 <sup>a</sup>	52.3 <sup>bc</sup>	33.7 <sup>c</sup>	9.2 <sup>b</sup>	0.36 <sup>a</sup>
Farm soil (0-5 cm depth)	5.5 <sup>a</sup>	51.1 <sup>bcd</sup>	35.8 <sup>bc</sup>	8.8 <sup>b</sup>	0.05 <sup>c</sup>
Mound	0.1 <sup>b</sup>	67.5 <sup>a</sup>	27.9 <sup>c</sup>	5.8 <sup>c</sup>	0.03 <sup>c</sup>
<i>SE</i>	1.28	2.72	2.42	0.71	0.06
<i>Cropping system</i>	*	**	**	*	-

\*Significant at  $P < 0.05$ ; \*\* significant at  $P < 0.01$ ; SE=Standard error of means; all treatments except mound had crop residue ( $2 \text{ t ha}^{-1}$ ) added; values in the same column followed by the same letter are not significantly different at  $P < 0.05$ ; LM=large macroaggregates ( $>2000 \mu\text{m}$ ), SM= small macroaggregates ( $>250 < 2000 \mu\text{m}$ ), Mi=microaggregates ( $>53 < 250 \mu\text{m}$ ), S+C=silt+clay ( $<53 \mu\text{m}$ ), POM= particulate organic matter

#### **4.4 Discussion**

##### **4.4.1 Crop residue disappearance**

The rate of disappearance of maize stover applied as crop residue in Nyabeda, western Kenya, was fast, with 85% disappearance in 3.5 months, compared to, for example, 50% disappearance of maize residue over a similar period during a summer season in Zimbabwe (Nhamo 2007). The combination of warm and sub-humid conditions in Kenya, compared to the rather dry conditions in Zimbabwe, could have led to the faster residue disappearance. Even so, with regular irrigation, fast residue disappearance can occur in the dry areas, such as the disappearance of about 80% of millet straw over 3.5 months reported for Niger (Fatondji 2002). The rate of residue disappearance in general depends on several factors, including the litter quality itself, the macro- and microclimate at the site, and the density and activity of soil macro- and mesofauna involved in breaking down the residues into debris, which also differ from site to site (Couteaux et al. 1995; González and Seastedt 2001; Pouyat and Carreiro 2003).

Residue disappearance was always greater in 5 mm mesh-sized litterbags than in 1 mm litterbags, indicating the crucial role of macro- and mesofauna in breakdown of added residue, as also reported by Kurzatkowski et al. (2004) for tropical rainforest and Irmiler (2000) and Wachendorf et al. (1997) for temperate forests, and by others in other ecosystems (Tian et al. 1997). Crop residue disappearance results in increased exposure of surface soil, and hence increased soil water evaporation and variability of surface temperature, and can potentially reduce the expected yields in conservation tillage systems. Thus, additional strategies to supplement crop residue are needed in western Kenya. As residue losses in presence of macro- and mesofauna were similar for surface-placed and buried crop residue, farmers can apply such low-quality residue as maize stover on the surface to avoid reduced crop yields due to nutrient immobilization, which is also the easier and cheaper way than incorporation of residues.

The large role of soil macro- and mesofauna in the fast disappearance of applied crop residue in western Kenya is not unusual, as similar results are reported by other authors (Kurzatkowski et al. 2004; Mando and Brussard 1999). Termites are especially very predominant at the study site, as also in other areas in Africa (Arshad et al. 1988; Maduakor et al. 1995). The greater contribution of macro- and mesofauna to the disappearance of surface-placed crop residue than to that of buried material could be

due to greater accessibility of surface-placed organic matter to the fauna. Overall, the decreasing contribution of macro- and mesofauna to residue disappearance with time reflects the changing residue quality. The decline in residue disappearance also coincided with the time when leaves fallen from growing maize plants provided alternative feed to soil organisms. Similar patterns in the overall residue loss rate of surface-placed and buried residue, regardless of a significantly lower contribution of macro- and mesofauna to the loss of buried residue, can be attributed to increased soil moisture (Steiner et al. 1999) and maximized residue-soil contact for buried residue. These have effects on greater residue accessibility to, and activity of soil fauna (that can pass through 1 mm mesh size), and their contribution may lead to the faster disappearance of buried crop residue.

Crop residues undergo chemical transformations during the cropping cycle due to the effects of soil organisms and rainfall/water that leach out soluble nutrients (Heal et al. 1997; Mun and Whitford 1998). The increase of N and P concentrations in crop residue with time is not unique to our study, and is attributed to importation in organics (saliva, feces) by termites. In their study, McTiernan et al. (2003) found an 80% increase in *Pinus sylvestris* litter %N by the time of 50% litter loss in different sites across Netherlands to south Spain and, similar to other authors (Wachendorf et al. 1997), attributed it to input by microbial processes. Dissolution of potassium (K), a particularly soluble nutrient, in rainwater and leaching out from the crop residue could be the reason for the sharp decrease in K content in the first month. This is consistent with the findings of other researchers. For example, Schroth et al. (1992) found that up to 80% of K in *Cajanus cajan* residues had decreased in only 11 days in the sub-humid zone of Togo, while Fatondji (2002) reported an 80% release of K from millet residues within 32 days after application under Sahelian conditions in Niger. The increase in lignin with residue loss has been reported previously for different places (Cousteaux et al. 1995; Mun and Whitford 1998; Wedin et al. 1995). The lignin increase in the first month, when also at least 50% of crop residue disappeared, is an indication that termites selected low-lignin organic compounds of the residue. We observed that termites attacked first the leaves of the applied maize stover, followed by inner stalk (mainly soft ground tissues), while the outer parts of the stalk (epidermis and hypodermis) were rarely attacked. Within the same zone, Kooyman and Onck (1987b) found that termites

of the genus *Microtermes* attacked only the inner soft tissues of the maize stalk. Thus, although termites generally prefer recalcitrant organic materials (Ouédraogo et al. 2004) such as maize stover, they are selective on the specific plant tissues. Ash content increases with residue loss due to contamination with minerals through movements of soil fauna (McTiernan et al. 2003) and soil water. The unusually sharp and significant increase in ash content in the fourth month, when only 15% of initial residue was present, was unexpected and we could not establish with confidence whether it was real or due to artifacts, as the findings were based on one season data only.

#### **4.4.2 Termite activity**

Plants modify surface and belowground soil microclimate, and they influence the activity of soil fauna such as termites (Martius et al. 2004). Although we attributed low termite activity under soybean to the presence of ants based on anecdotal evidence, reduced termite activity was previously associated with improved residue quality (Tian et al. 1995), and this could also apply to our soybean plots due to addition of high-quality soybean litter (see also section 4.4.1; discussion of termite preference of recalcitrant material). Below the soil surface, the slightly elevated termite activity under soybean, similar to the finding with crop residue disappearance, could be related to microclimate effects of the soybean canopy cover (it can reach nearly 100% in less than 3 months). Such canopy cover effects include extended periods with increased soil moisture, reduced solar radiation and temperature variations, which also increase fauna diversity (Kurzatkowski et al. 2004; Martius et al. 2004).

Highest surface termite activity in plots without crop residue application, as also observed in Uganda (Sekamatte et al. 2001), shows that application of crop residue can reduce damage to standing maize stems. This is because the applied crop residue serves as feed for soil fauna such as termites that otherwise feed on the standing maize stems as in the control treatment. However, long-term assessment is required, since regular crop residue application could in the long-run create favorable conditions for termites to attack maize.

#### **4.4.3 Aggregate characteristics of termite-molded surface sheeting and mound soil**

Termite-molded gallery/sheeting soil is more carbon-enriched than other farm soil, as also observed elsewhere (Brauman 2000; Kooyman and Onck 1987b), due to mixing of soil with termite saliva during soil collection, transportation and molding (Sarcinelli et al. 2009; Zaady et al. 2003). The extent of the carbon enrichment depends also on the quality of the surrounding soil (Fall et al. 2001). The higher carbon enrichment in the legume-based cropping systems than in the continuous cereal system was greater than the slight difference in total soil carbon (1.38% vs. 1.34%, data not shown), suggesting that termites could have selected carbon-rich soils. The lower carbon content of nesting/mound material could be due to importation of low carbon soil from lower parts of the profile during nest construction (Maduakor et al. 1995), with little contribution of termite feces (Fall et al. 2001), e.g., Macrotermitinae use feces exclusively for construction of fungus combs (Kooyman and Onck 1987b). Previously, mound material (possibly outer walls) of fungus-feeding *Macrotermes* (of the same Macrotermitinae subfamily as termites in the site of the current study) was observed to have lower carbon than the surrounding soil in Kenya (Arshad et al. 1988) and in Nigeria, and this was attributed mainly to the termites feeding on carbon-depleted subsoil (Maduakor et al. 1995). However, since mound enrichment depends on the termite species involved, age and part of the nest sampled, other studies have reported greater enrichment of carbon (and also other nutrients and cation exchange capacity) in termite-molded mound soil than in surrounding soils (Rückamp et al. 2009a; Rückamp et al. 2009b; Sarcinelli et al. 2009). Thus, whereas outer mound walls can have decreased soil carbon relative to surrounding soil, termite-molded surface sheeting soil contributes to nutrient enrichment of the farm soil and could benefit farmers through increased yields, derived from long-term improvement of soil nutrient status.

It is generally agreed by scientists that termites contribute to soil aggregation and thus soil structure (Brauman 2000; Kooyman and Onck 1987b), and this is confirmed by the highest macroaggregation in nesting material observed in this study. Jungerius et al. (1999) and Sarcinelli et al. (2009) showed that termite mounds contained mainly 1.0 mm-sized compound aggregates composed of basic aggregates (about 0.5 mm) from the subsoil. This is consistent with the finding of the current study



that up to 68% of the mound soil was in the  $>250<2000\ \mu\text{m}$  size class. The small fraction of large macroaggregates ( $>2000\ \mu\text{m}$ ) for mound soil in our study can be related to the difficult transport of large aggregates upwards through the tunnels, but the carrying capacity of termites, determined by their mandible size, had also been suggested (Jungerius et al. 1999). The cementation by saliva and other termite body fluids (Sarcinelli et al. 2009) can explain the small amount of free silt+clay in these mound soils. Thus, although formed preferentially from clay particles (Fall et al. 2001; Fall et al. 2004), mounds, such as those of fungus-growing *Macrotermes*, have the clay structurally stabilized in the soil macroaggregates. When the macroaggregates finally disintegrate, the microaggregates that are well-stabilized due to cementation by termite saliva and sticky body fluid persist in the soil (Jungerius et al. 1999) and are common in places with past termite activities (Trapnell and Webster 1986), evidence that termite-molded aggregates result in a long-term influence on the soil structure.

Unlike the termite mound/nest soils, termite-molded surface sheeting soils are usually loosely held, disintegrate readily and slake easily into individual aggregates (Jungerius et al. 1999). Surprisingly, the aggregates are of the same size distribution and are affected similarly by management as the aggregates of the bulk farm soil (see also Chapter 2) implying that, in our case, the soils were scavenged and used in the gallery/sheeting without breaking them further. It has been shown that the soils used in sheeting by Macrotermitinae are mainly in the  $150\ \mu\text{m}$  to  $450\ \mu\text{m}$  size class range (Kooyman and Onck 1987a, b), which corresponds to the microaggregate and small macroaggregate sizes constituting the largest proportions of sheeting soils in our study. The presence of large macroaggregates in termite-molded surface sheeting soils, unlike in the mound soils as shown earlier, indicates that the macroaggregates were gathered from the surface soil, as it is difficult to pass large aggregates through the tunnels. Because they are water-stable, it appears that such termite-molded aggregates could remain intact for a long time when distributed within the bulk soil. Earlier, macroaggregates ( $>250<2000\ \mu\text{m}$ ) in the bulk soil had been shown to increase with density of termites at our study site (Ndabamenye 2006). Perhaps, because of enrichment with organic glues, termite-molded sheeting soil easily bonds with other soil particles to form stable macroaggregates. Thus, termites are important in soil

aggregation, but further studies are needed to fully understand whether termite gallery materials contribute to farm soil aggregation more than similarly sized bulk soil.

Although the termite-molded surface sheeting soils were not as aggregated as the mound soil, their greater carbon enrichment reported in our study, as well as enrichment in other nutrients and increased CEC (Kooyman and Onck 1987b), can lead to a substantial agronomic impact, especially because large amounts of such surface termite-molded gallery (sheeting) soils of up to 1300 kg ha<sup>-1</sup> are molded annually (Kooyman and Onck 1987b).

#### **4.5 Conclusions**

The rate of crop residue disappearance in sub-humid western Kenya is fast, and up to 60% of initial residue is lost within 50 days after application. Before 85% of residue disappearance occurs, macro- and mesofauna contribute between 56% and 90% to the disappearance of crop residue, which supports the hypothesis that macro- and mesofauna contribute more to residue disappearance than other biotic and abiotic factors. The contribution of macro- and mesofauna to residue disappearance is greater for surface-placed (by 10 to 50%) than for buried residue, and it decreases with time due to changes in residue quality. Termites sequentially first attacked the leaves of the applied maize stover, then inner stalk material (ground tissues), while the outer stalk was rarely attacked. As hypothesized, termite-molded surface sheeting soils have 11% to 26% higher carbon content, but contrary to our second hypothesis, they have soil aggregate size fractions similar to those of bulk farm soil. Mound soils have similar carbon content but higher proportion of macroaggregates (by 32%) relative to bulk soil. Data from other studies suggest a high turnover and hence, a quantitative importance of these effects for cropping system management, but this would need to be ascertained more precisely in future studies.

## **5 EFFECTS OF TILLAGE AND CROP RESIDUE ON SOYBEAN NITROGEN FIXATION**

### **5.1 Introduction**

Relying on the biological nitrogen fixation (BNF) of grain legumes is a strategy to ease the burden that commercial fertilizers exert on poor farmers. In Africa, fertilizer use is estimated at 8 kg ha<sup>-1</sup>, which is only 10% of the world's average (Maatman et al. 2008). Stoorvogel et al. (1993) pointed out gross annual nutrient mining in sub-Saharan Africa (SSA) averaging 22 kg N ha<sup>-1</sup>, but up to 100 kg N ha<sup>-1</sup> in some cases. The unique socio-economic conditions and wide-spread rural poverty in sub-Saharan Africa limit access to mineral fertilizers, inducing the search for cheap sources of fertilization, such as nitrogen fixation by grain legumes. Soybean has been produced in Kenya for several years on a small-scale, but demand for proteins and alternative oil has renewed interest in its production. A first evaluation of soybean germplasm took place as early as 1993 (Nassiuma and Wasike 2002), but promiscuous soybean varieties (varieties that do not require inoculation with specific *Rhizobium*) have only recently been introduced to western Kenya for soil fertility improvement, through the Tropical Soil Biology and Fertility institute of CIAT (TSBF-CIAT), and their performance is being tested in various agro-ecological zones.

The amount of nitrogen fixed by grain legumes such as soybean is affected by the degree of colonization by soil rhizobia (Mabood et al. 2006) as well as by their interaction with other biological, physical and chemical properties of the soil (Goss and de Varennes 2002; Rebařka et al. 1993) and by weather (Streeter 2003). These factors, except weather, are themselves influenced by management practices such as tillage and crop residue application as well as by the spatial-temporal arrangement of crop components. Reduced soil disturbance, for example, improves soil physical parameters such as structure (this study), but also the chemical and biological parameters that affect nitrogen fixation. Soil moisture and temperature, for example, which are influenced by tillage and crop residue, affect biological nitrogen fixation (Salvagiotti et al. 2008). Legumes such as soybean that transport N fixed from roots to shoots in the form of ureides (allantoin and allantoic acid) are particularly susceptible to water stress (Sinclair et al. 2007), and conservation practices involving reduced tillage and surface crop

residue application are expected to positively influence nitrogen fixation. Also, crop residue management affects decomposition of the residue and rates of N-release to the soil pool, thus affecting nitrogen fixation (Giller and Cadisch 1995; Peoples and Craswell 1992). Small-scale crop production in eastern Africa takes place mostly without mulching, but about 2 t ha<sup>-1</sup> of low-quality crop residue can be applied or just remain from the previous cereal crop. It is not known to which extent such low-quality crop residue mulch, in quantities affordable by small-scale farmers, affects proportion and total amount of nitrogen fixed by soybean in eastern Africa. The effect of crop residue on nitrogen fixation in grain legumes has been investigated on sandy soils (Rebafka et al. 1993), but no work on tropical African Ferralsols could be found.

Within smallholder production systems, soybean is produced in rotation or intercropping with cereals. Quantifying the contribution of soybean to the nitrogen balance in these systems under different tillage and residue management scenarios is important for optimization of BNF. Appreciable amounts of soybean leaves can be shed during the soybean crop cycle thus contributing to the N-balance in these systems. Peoples and Craswell (1992) noted that frequently, only standing shoot data and not leaf-fall data are available, and therefore net N-balances are underestimated in many studies. We could not find any N-fixation study dealing with African Ferralsols that took into account nitrogen contained in abscised litter. In addition, although much work has been published on soybean production and nitrogen fixation in western Africa (Dakora and Keya 1997; Osunde et al. 2003; Sanginga 2003), not much work was conducted in eastern Africa.

This study aimed to quantify nitrogen fixation of soybean under reduced and conventional tillage with different crop residue management in a sub-humid zone of western Kenya. We hypothesized that practicing reduced tillage combined with crop residue application leads to a greater proportion of nitrogen derived from the atmosphere and to greater total nitrogen fixed, compared to reduced tillage without crop residue application, and also compared to conventional tillage systems.

## **5.2 Materials and methods**

### **5.2.1 Site and soil type**

The study was conducted in a soybean-maize rotation and intercropping system on a tropical Ferralsol during the long rains in 2007 in Nyabeda, western Kenya. The experiment was established as a long-term conservation tillage trial in March 2003 (for details see Table 1.1). The experiment was set up with a factorial design involving tillage (reduced and conventional tillage), crop residue (CR) application (maize stover applied at 0 and 2 t ha<sup>-1</sup>) and cropping systems (legume-cereal intercropping and rotation). Under reduced tillage (RT), tillage was restricted to surface-scratching down to 3 cm depth to remove weeds, while conventional tillage (CT) involved tillage with hand hoes to about 10-15 cm depth as done by small-scale farmers. Soybean (*Glycine max* (L.) Merr) (TGX 1448-2E, locally known as SB20) was the legume used, except in the first season when common bean (*Phaseolus vulgaris*) was used. Maize (*Zea mays* L.) was the cereal crop. Legume residues were left on the respective plots after harvest. Nitrogen in form of urea was applied at 0 and 60 kg N ha<sup>-1</sup>, and P was applied at 0 and 60 kg P ha<sup>-1</sup> as triple super phosphate (TSP). All the treatments received a basal application of 60 kg K ha<sup>-1</sup> as muriate of potash.

### **5.2.2 Soybean nodulation and biomass assessment**

Soybean nodulation was investigated at peak biomass in four different cropping seasons between September 2003 and August 2005. One quadrant measuring 0.75 m by 0.75 m was placed on each diagonal of the plot, aboveground shoots cut and roots carefully excavated, and nodules sorted out by hand. After washing to soil-free, nodules were counted, fresh weights determined, and dry weights determined after oven-drying at 60°C for 48 hours.

### **5.2.3 Soybean in-season leaf fall**

Six plastic nets (traps), each measuring 50 cm by 75 cm, were laid 5 cm above the soil surface at 6 weeks after planting to collect soybean litter-fall. The leaves and flowers falling in the litter traps were collected on a weekly basis starting from 61 days after planting and twice weekly from 83 days after planting. Weight of the collected leaves and flowers was taken before and after drying at 60°C for 48 hours. At the end of the

season, soil-free leaves and flowers for each treatment were bulked by replicate and analyzed for N content. Soybean leaf and flower debris mixed with soil were also taken, and a subsample ashed at 550°C for 5 hours to determine the mineral content that was used to estimate leaf and flower biomass in the biomass-soil mixture.

#### **5.2.4 Microplots and $^{15}\text{N}$ dilution**

Microplots measuring 2 m by 3 m and 3 m by 3 m were established in April 2007 within soybean-maize rotation and intercropping systems, respectively. Urea ( $\text{CO}(^{15}\text{NH}_2)_2$ ), with atom %  $^{15}\text{N}$  of 5.30 $\pm$ 0.05, was applied at 6 g and 9 g in soybean-maize rotation and intercropping systems, respectively, split applied  $\frac{1}{2}$  at planting and  $\frac{1}{2}$  at 50 days after planting. The target application rate was 10 kg N ha $^{-1}$ . The microplots were planted with nitrogen fixing (F) soybean and non-nitrogen fixing reference (NF) maize plants. No inoculation was done, and soybean was expected to self-inoculate with native soil bacteria. Harvesting of the microplots was done at 115 days after planting (at physiological maturity) when the soybean pods turned yellow-to-brown. For aboveground biomass, four maize plants were sampled from each reference plot and their stems, pods and root biomass taken. For soybean, sampling was done for all plants within a distance of 50 cm from the two ends of the microplot (area of 2 m $^2$  for rotation and 4 m $^2$  for intercropping). Fresh weights for all samples were taken in the field. All aboveground biomass was oven-dried at 60°C for 48 hours before taking dry weights. Due to oven availability, some of the samples were air-dried for 2 days before the oven-drying, and this was not expected to result in significant loss in N content.

Root biomass in microplots was assessed in two quadrats of 0.50 m by 0.75 m instead of the whole microplot to avoid disturbance in the reduced tillage plots. The area was dug out to 15 cm depth using a hand-hoe and the roots sorted out by hand. Root fresh weights for all root samples were taken in the laboratory after washing to soil-free with de-ionized water, over a 0.5 mm sieve. The samples were put in the oven immediately after washing and dried at 60°C for 48 hours.

Laboratory analysis for total N and atom %  $^{15}\text{N}$  was done for each of the plant parts (stems and leaves, pods, grains, and roots) at the Institute of Plant Nutrition Laboratory in Bonn, Germany, using an isotope ratio mass spectrometer ANCA-SL (PDZ Europa, Cheshire, UK, 1998). Soils sampled from soybean-maize rotation,

soybean-maize intercropping and continuous maize at the beginning of season 9 (in March 2007) were also analyzed for  $\delta^{15}\text{N}$  concentration. Litter N was analyzed by wet oxidation based on Kjeldahl digestion with sulphuric acid (ICRAF 1995) in the ICRAF laboratories in Nairobi, Kenya, and its  $^{15}\text{N}/^{14}\text{N}$  ratio was assumed to be similar to that of stems plus leaves at physiological maturity.

### 5.2.5 Calculations to quantify N fixation

The following calculations, as given by IAEA (2001), were used to calculate the amounts of nitrogen fixation:

$$\%NDfA = \left( 1 - \frac{\%NDfF_F}{\%NDfF_{NF}} \right) \times 100 \quad (5.1)$$

where  $\%NDfF = \frac{Atom\%^{15}N_{Excess\ plant}}{Atom\%^{15}N_{Excess\ Fertilizer}} \times 100$  and the subscripts F and NF refer to fixing and non-fixing plants, respectively. Atom %  $^{15}\text{N}$  excess for the fertilizer and plant were determined by subtracting the atmospheric natural abundance value of 0.366303 from the measured atom %  $^{15}\text{N}$  concentrations.

Calculation of total plant %NDfA was by weighting the %NDfA of the specific plant parts based on their masses according to the formula,

$$\%NDfA_{Total} = \frac{(\%NDfA_a \times M_a) + (\%NDfA_b \times M_b) + (\%NDfA_c \times M_c)}{M_a + M_b + M_c} \quad (5.2)$$

where subscripts a, b and c represent stems and leaves, grain, and husks, respectively, and M is the weight of the harvested parts.

The ratio of nitrogen derived from the fertilizer (NDfF) and from soil (NDfS) for the soybean was assumed to be the same as that for the reference maize crop, and thus the proportion of nitrogen derived from soil for the fixing crop was calculated as:

$$\%NDfS_F = NDfF_F \times \frac{\%NDfS_{NF}}{\%NDfF_{NF}} \quad (5.3)$$

The amount of fixed nitrogen was derived by multiplying % NDfA with total N content of the fixing soybean plant.

Nitrogen balances were calculated by subtracting total N contained in harvest products, mainly soybean and maize grain, from total N fixed. For rotation, the balances refer to two cropping cycles, while intercropping covers one season. Balances in the two cropping seasons were compared under the assumption that the balances in the intercropping system could be doubled to also reflect two seasons.

#### **5.2.6 Data analysis**

Data were analyzed using SAS version 9.1 statistical software for Windows. The “Mixed” model procedure was used for analysis of variance between treatments least square means, allowing for analysis of random effects of the replicate, tillage practice and applied crop residue.

### **5.3 Results**

#### **5.3.1 Nodulation**

Nodulation was generally lower in the intercrop than in the rotation (Table 5.1) and was attributed to competition effects of the superimposed maize in the intercrop, as opposed to the monocropped soybean in rotation. Besides that, nodulation was always lowest ( $P < 0.05$ ) under ‘reduced tillage minus crop residue’ (e.g., -27% to -53% for nodule weight). However, in ‘reduced tillage plus crop residue’, nodulation was in most cases as high as in conventional tillage treatments. Application of crop residue resulted in large increases (15-79%) in nodulation in reduced tillage, but only slight increases (5-17%) in conventional tillage. In the reduced tillage system, the increases in nodulation following crop residue application were significant ( $P < 0.05$ ) for biomass (in rotation) and nodule numbers (in intercrop). The low nodulation in ‘reduced tillage minus crop residue’ may be related to surface crusting (this was visually observed and it was more pronounced in this treatment than in others), which could have resulted in increased losses in runoff of the applied phosphorus. This was implied by the low soil available P of 10 mg P kg<sup>-1</sup> soil in ‘reduced tillage minus crop residue’, compared to over 22 mg P kg<sup>-1</sup> soil in the other treatments (data not shown; these values were taken two years later, at the time of the study with the <sup>15</sup>N isotope).



Table 5.1. Average nodule number ( $\log_{10}$  transformed) and weight ( $\text{kg ha}^{-1}$ ) at podding as observed over 3 seasons between 2003 and 2005 in Nyabeda, western Kenya

<i>Treatment</i>	<i>Nodule weight</i> --( $\text{kg ha}^{-1}$ )--	<i>Number of nodules m<sup>-2</sup></i>
Intercrop		
Conventional tillage -CR	24.2 <sup>ba</sup>	2.2 <sup>a</sup>
Conventional tillage +CR	27.2 <sup>a</sup>	2.4 <sup>a</sup>
Reduced tillage -CR	17.6 <sup>c</sup>	1.9 <sup>b</sup>
Reduced tillage +CR	20.3 <sup>cb</sup>	2.3 <sup>a</sup>
<i>SED</i>	2.68	0.08
<i>Tillage</i>	*	-
<i>Crop residue</i>	-	**
<i>Tillage x crop residue</i>	-	*
Rotation		
Conventional tillage -CR	37.2 <sup>a</sup>	2.3 <sup>a</sup>
Conventional tillage +CR	43.6 <sup>a</sup>	2.4 <sup>a</sup>
Reduced tillage -CR	20.3 <sup>b</sup>	1.9 <sup>b</sup>
Reduced tillage +CR	36.4 <sup>a</sup>	2.3 <sup>ab</sup>
<i>SED</i>	5.38	0.14
<i>Tillage</i>	*	-
<i>Crop residue</i>	**	**

CR=crop residue, SED=standard error of differences of means, values in the same column and cropping system followed by the same letter are not significantly different at  $P=0.05$ , \*significant effect at  $P<0.05$ , \*\*significant effect at  $P<0.01$ , -no significant effect

The effects of fertilizer N and P on nodulation in a rotation system were analyzed (Figure 5.1). Application of P alone (-N+P) resulted in higher nodule weight ( $P<0.05$ ); it was 3 to 16 times higher than the control (-N-P), and 0.5 to 3 times higher than when N and P were given together (+N+P). Lower nodulation with application of

chemical inorganic N fertilizer was expected due to lower plant demand for fixed-N. Since application of P without N was the most promising strategy, soybean nitrogen fixation was investigated further only under this system, using the  $^{15}\text{N}$  dilution method. Seasonal differences in nodulation are explained by amount and distribution of seasonal rainfall; the amount of seasonal rainfall was low in the short rains (SR) 2003 season, and in long rains (LR) 2004 and SR 2004 seasons, rainfall amount was poorly distributed compared to LR 2005 season.

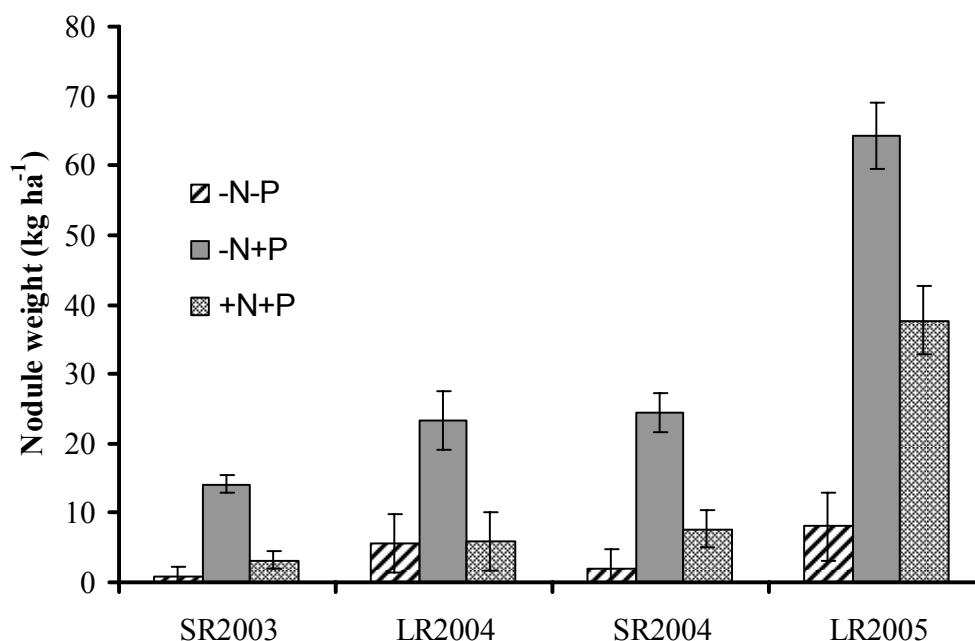


Figure 5.1. Effect of fertilizer N and P on soybean nodule weight in different seasons in Nyabeda, western Kenya, within soybean-maize rotation system. LR=long rainy season (usually March to August), SR=short rainy season (usually September to January), nitrogen and phosphorus were each applied at 60 kg N ha<sup>-1</sup> in plots indicated +N and +P, error bars are least significant differences

### 5.3.2 Soybean below and aboveground biomass

Tillage and crop residue had no significant effect on products harvested at physiological maturity, except for litter-fall in the rotation system; here the effect was greater (by 29 to 43%;  $P < 0.05$ ) in 'reduced tillage minus crop residue' than in the other treatments (Table 5.2). The greater litter-fall was in the same 'reduced tillage minus crop residue' treatment that also had lower nodulation, perhaps due to combination of nutrient and water stress, which could induce early leaf abscission in this treatment. The early

induced leaf-fall was confirmed at harvest maturity where, as opposed to physiological maturity, litter-fall was similar across the treatments (0.96 to 1.10 t ha<sup>-1</sup> in the rotation system, also Figure 5.2). In the intercropping system, soybean grain yield in ‘reduced tillage minus crop residue’ was about 50% of that with crop residue application (though not significant) and this was attributed to water stress during the grain-filling stage, as only 34 mm rainfall was received between 72 and 106 days after planting. Competition for water among the soybean and the intercropped maize likely aggravated the water stress in reduced tillage in the absence of crop residue, relative to similar treatment in rotated soybean. The litter-fall at physiological maturity (Table 5.2) on average constituted about 64% of the total litter-fall observed up to harvest, both for intercropping and rotation (Figure 5.2). The first leaf abscission occurred about 60 days after planting, followed by a 3-week lag phase after which leaf-fall increased rapidly; this can be related to the physiological stage of the soybean.

Table 5.2. Soybean yield (t ha<sup>-1</sup>) at physiological maturity as observed in Nyabeda, western Kenya, March-August 2007 cropping season

<i>Treatment</i>	<i>Grain</i>	<i>Stems+leaves</i>	<i>husks</i>	<i>Litter-fall</i>	<i>Root</i>
Intercrop ---t ha <sup>-1</sup> ---					
Conventional tillage -CR	0.64	1.19	0.52	0.52	0.24
Conventional tillage +CR	0.50	1.19	0.51	0.59	0.24
Reduced tillage -CR	0.35	0.96	0.44	0.43	0.18
Reduced tillage +CR	0.71	1.18	0.49	0.45	0.22
<i>Average</i>	<i>0.55</i>	<i>1.13</i>	<i>0.49</i>	<i>0.50</i>	<i>0.22</i>
<i>SE</i>	<i>0.171</i>	<i>0.270</i>	<i>0.065</i>	<i>0.053</i>	<i>0.035</i>
Rotation---t ha <sup>-1</sup> ---					
Conventional tillage -CR	1.09	1.91	0.53	0.53	0.36
Conventional tillage +CR	1.20	2.15	0.48	0.59	0.43
Reduced tillage -CR	1.12	2.18	0.50	0.76	0.32
Reduced tillage +CR	1.25	2.03	0.52	0.57	0.33
<i>Average</i>	<i>1.16</i>	<i>2.07</i>	<i>0.51</i>	<i>0.61</i>	<i>0.36</i>
<i>SE</i>	<i>0.171</i>	<i>0.213</i>	<i>0.049</i>	<i>0.080</i>	<i>0.039</i>
<i>Tillage x CR</i>				*	

SE= standard error, CR= crop residue, \*significant effect at P<0.05

### 5.3.3 Nitrogen derived from the atmosphere (NDfA)

Distribution of  $^{15}\text{N}$  varied significantly between plant parts, with the highest concentration being found in roots, followed by stems and leaves, and similar values in husks and grain (Table 5.3). Such distribution was expected due to fractionation of  $^{15}\text{N}$  and  $^{14}\text{N}$  isotopes during transfer and reallocation of N-containing compounds to different plant parts in response to demand. The relative difference in atom %  $^{15}\text{N}$  between the fixing and non-fixing (reference) plants is used for determining the amount of fixed N. The atom %  $^{15}\text{N}$  values in the reference plant were 0.7%, 15%, 15% and 18% higher than in the fixing plant for roots, grain, husks, and stems and leaves, respectively. Thus, differences in root  $^{15}\text{N}$  levels between fixing and non-fixing crops were small, suggesting that little of the fixed N was held in the roots. The roots were thus excluded from further consideration in the assessment of BNF. Total nitrogen concentration was highest in grain and lowest in roots for both fixing and non-fixing plants. Soybean grain %N was greatly affected by cropping system (5.9% in intercrop and 6.1% in rotation;  $P < 0.01$ ). Soil atom %  $^{15}\text{N}$  content was 0.36925 ( $\delta^{15}\text{N}$  from 7.8-8.2‰) and was not affected by either tillage, crop residue or cropping systems (data not shown).

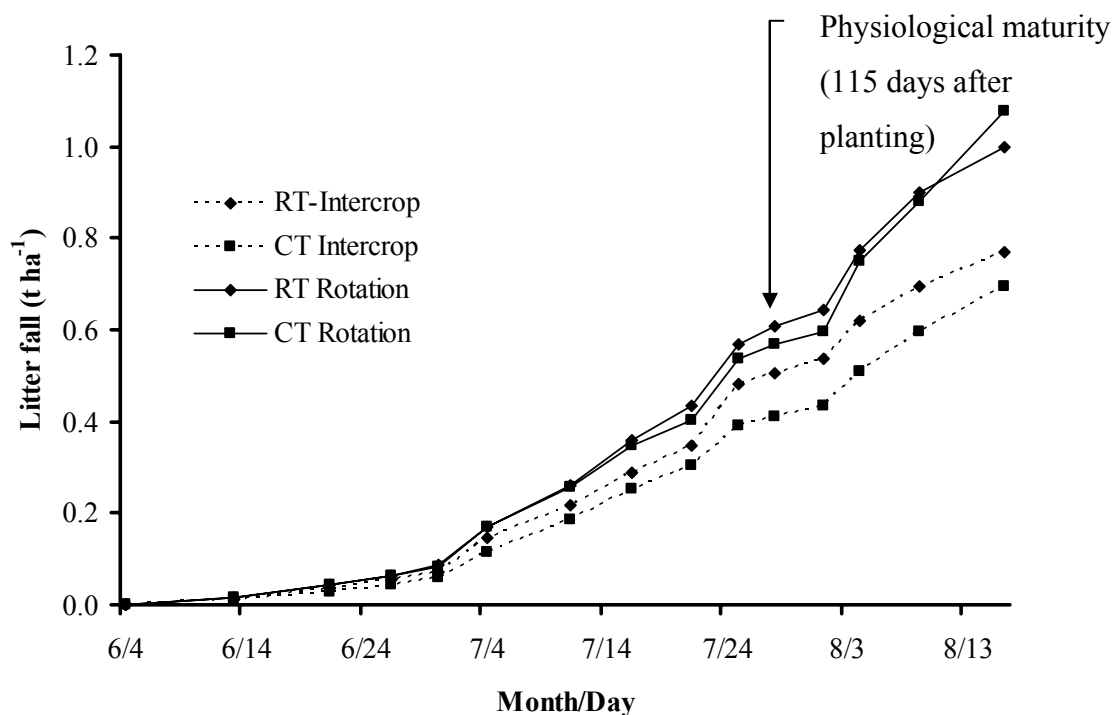


Figure 5.2. Soybean litter-fall in soybean-maize intercropping and rotation systems in Nyabeda, western Kenya, March-August 2007 cropping season. Arrow shows the date of sampling at physiological maturity, RT=reduced tillage, CT= conventional tillage

Table 5.3. Isotope  $^{15}\text{N}$  and total N concentration in different fixing and non-fixing plant parts in Nyabeda, western Kenya, March-August 2007 cropping season

Crop Part	Fixing plant		Reference plant	
	atom % $^{15}\text{N}$	% N	atom % $^{15}\text{N}$	% N
Stems and leaves	0.4333 <sup>b</sup>	1.33 <sup>b</sup> (1.19-1.48)	0.5127 <sup>b</sup>	0.51 <sup>b</sup> (0.45-0.57)
Husks	0.4252 <sup>cb</sup>	1.42 <sup>b</sup> (1.28-1.57)	0.4900 <sup>c</sup>	0.51 <sup>b</sup> (0.45-0.57)
Grain	0.4210 <sup>c</sup>	6.01 <sup>a</sup> (5.87-6.15)	0.4819 <sup>c</sup>	1.26 <sup>a</sup> (1.20-1.32)
Roots	0.5363 <sup>a</sup>	1.04 <sup>c</sup> (0.89-1.18)	0.5401 <sup>a</sup>	0.40 <sup>c</sup> (0.34-0.46)
Abscised leaves	-	1.60 (1.39-1.94)	-	-
SE	0.00513	0.072	0.00613	0.031

Numbers in bracket are lower and upper confidence limits, respectively; abscised leaves were not included in analysis of variance for total N, as only few bulked representative samples had been determined; SE=standard error.

In general, the proportion of soybean N derived from biological fixation ranged from 42 to 57% in the intercropping and 47 to 65% in the rotation system (Table

5.4). The slightly decreased %NDfA in the intercropping system (of 50% versus that of 54% NDfA in rotation), was due to the possible depressing effects of reduced light and competition of soil water and nutrients by the superimposed maize in the intercropping system. This was not consistent with nodulation results (greater nodulation in rotation than intercropping). The expected differences in %NDfA, unlike nodulation, could have been diluted, as the fixed-N in the rotation system was distributed also to greater plant biomass.

Regardless of the cropping system, practicing reduced tillage increased ( $P<0.05$ ) total (and also grain) %NDfA (a difference of 9% NDfA) relative to that in conventional tillage, and in contrast to nodulation observed at least 2 years earlier. It is possible that soil biological conditions, for instance, improved in reduced tillage during the two years resulting in better conditions for higher BNF compared to conventional tillage. Increased %NDfA in reduced tillage resulted in decreased nitrogen derived from the soil (NDfS) of 42% in contrast to 51% in conventional tillage ( $P<0.05$ ; data not shown).

There was no significant effect of crop residue on %NDfA, but interaction of crop residue with tillage had a significant effect ( $P<0.05$ ). In the reduced tillage, the response to crop residue varied; crop residue application resulted in a large increase ( $P<0.05$ ), from 47 to 65% NDfA in the rotation system, as expected, but not in the intercropping system. In the intercropping system, the fixed N in 'reduced tillage plus crop residue' was distributed to the greater plant biomass than in 'reduced tillage minus crop residue' (as reported earlier, e.g., for grain), hence the unexpected %NDfA in these two treatments. Application of crop residue in conventional tillage slightly decreased %NDfA in both cropping systems (a difference of 4% and 10% NDfA in intercropping and rotation systems, respectively), attributable to possibly increased available N following mineralization of the incorporated residue.

The proportion of nitrogen derived from fertilizer (%NDfF) was small, being only 1% in soybean grain and 1.3% in biomass and husks (data not shown). This represents a low nitrogen recovery of only about 5%, which can be attributed to nitrogen losses through denitrification, as rainfall was also relatively high during the first two months (450 mm rainfall) of the season under consideration.

Table 5.4. Nitrogen derived from the air (%NDfA) from soybean intercropped and rotated with maize in Nyabeda, western Kenya, March-August 2007 cropping season

<i>Treatment</i>	<i>Grain</i>	<i>Stems and leaves</i>	<i>Husks</i>	<i>Total<sup>#</sup></i>
Intercrop----%NDfA----				
Conventional tillage -CR	44.6 <sup>a</sup>	54.6 <sup>a</sup>	36.3 <sup>a</sup>	46.3 <sup>a</sup>
Conventional tillage +CR	42.6 <sup>a</sup>	43.1 <sup>a</sup>	40.9 <sup>a</sup>	42.0 <sup>a</sup>
Reduced tillage -CR	60.4 <sup>a</sup>	58.0 <sup>a</sup>	53.8 <sup>a</sup>	56.5 <sup>a</sup>
Reduced tillage +CR	57.1 <sup>a</sup>	57.2 <sup>a</sup>	47.9 <sup>a</sup>	54.4 <sup>a</sup>
<i>average</i>	<i>51.2</i>	<i>53.2</i>	<i>44.7</i>	<i>49.8</i>
<i>SE</i>	<i>6.5</i>	<i>8.28</i>	<i>5.32</i>	<i>5.72</i>
Rotation----%NDfA----				
Conventional tillage -CR	55.6 <sup>ab</sup>	59.7 <sup>a</sup>	58.8 <sup>ab</sup>	57.6 <sup>ab</sup>
Conventional tillage +CR	41.4 <sup>b</sup>	52.3 <sup>a</sup>	43.6 <sup>c</sup>	47.0 <sup>b</sup>
Reduced tillage -CR	48.8 <sup>b</sup>	45.6 <sup>a</sup>	54.3 <sup>bc</sup>	46.7 <sup>b</sup>
Reduced tillage +CR	67.2 <sup>a</sup>	65.0 <sup>a</sup>	67.6 <sup>a</sup>	64.9 <sup>a</sup>
<i>average</i>	<i>53.2</i>	<i>55.6</i>	<i>56.1</i>	<i>54.1</i>
<i>SE</i>	<i>4.9</i>	<i>6.56</i>	<i>7.25</i>	<i>4.61</i>
<i>Tillage x CR</i>	<i>*</i>		<i>**</i>	<i>**</i>
<i>SE<sup>§</sup></i>	<i>5.77</i>	<i>7.47</i>	<i>6.36</i>	<i>3.96</i>
<i>Tillage<sup>§</sup></i>	<i>*</i>			<i>*</i>
<i>Tillage x CR<sup>§</sup></i>				<i>*</i>
<i>Tillage x CR x cropping system<sup>§</sup></i>	<i>*</i>		<i>*</i>	<i>**</i>

<sup>#</sup>calculated by weighting with respective plant part mass recorded in Table 5.2; values in the same column and cropping system followed by the same letter are not significantly different at P<0.05; <sup>§</sup> shows effect across the two cropping systems; CR= crop residue; SE=standard error; \*significant at P<0.05; \*\*significant at P<0.01

#### 5.3.4 Nitrogen fixed

The amount of fixed N in soybean aboveground plant parts was 26-48 kg N ha<sup>-1</sup> in the intercropping and 53-82 kg N ha<sup>-1</sup> in the rotation system (Table 5.5), with the lower fixed N in intercropping attributed to the observed lower plant harvest biomass due to competition effects of the superimposed maize crop. Under both rotation and intercropping, practicing 'reduced tillage plus crop residue' led to the highest grain and biomass N as well as total fixed N when compared to conventional tillage and 'reduced tillage minus crop residue' treatments. Total fixed N in 'reduced tillage plus crop residue' was, for instance, higher than in the other treatments by at least 55% in the soybean-maize intercropping and 34% in the rotation system. The lowest total amount of fixed N was observed in the conventional tillage system, for both intercropping and rotation systems. Since the harvested crop biomass was largely the same among the treatments within a cropping system, the differences in amounts of fixed N are largely attributable to the differences in %NDfA.

As with %NDfA, application of crop residue increased (not significantly) the total amount of fixed N under reduced tillage by 34% and 64% in rotation and intercropping systems, respectively, but slightly suppressed the fixed N under conventional tillage (by 9% and 14% in rotation and intercropping, respectively). These effects can also be largely attributed to the differences observed in %NDfA shown earlier.

By the time of physiological maturity, the amount of nitrogen biologically fixed in shed leaves ranged from 3.7 to 5.8 kg N ha<sup>-1</sup>, representing about 54% of the total N (8.2 to 11.8 kg N ha<sup>-1</sup>) contained in leaf-fall by the time of physiological maturity.



Table 5.5. Nitrogen fixed (kg N ha<sup>-1</sup>) in soybean under intercrop and rotation with maize in Nyabeda, western Kenya, March-August 2007 cropping season

<i>Treatment</i>	<i>Grain</i>	<i>Biomass</i>	<i>Husks</i>	<i>Litter-fall</i>	<i>Total</i>
Intercrop----kg N ha <sup>-1</sup> ----					
Conventional tillage -CR	14.9 <sup>a</sup>	7.4 <sup>a</sup>	2.8 <sup>a</sup>	5.6 <sup>a</sup>	30.7 <sup>a</sup>
Conventional tillage +CR	12.3 <sup>a</sup>	6.8 <sup>a</sup>	3.5 <sup>a</sup>	3.8 <sup>a</sup>	26.3 <sup>a</sup>
Reduced tillage -CR	12.8 <sup>a</sup>	7.9 <sup>a</sup>	3.4 <sup>a</sup>	4.7 <sup>a</sup>	28.9 <sup>a</sup>
Reduced tillage +CR	28.2 <sup>a</sup>	12.2 <sup>a</sup>	3.5 <sup>a</sup>	3.7 <sup>a</sup>	47.5 <sup>a</sup>
<i>Average</i>	<i>17.0</i>	<i>8.6</i>	<i>3.3</i>	<i>4.5</i>	<i>33.4</i>
<i>SE</i>	<i>7.97</i>	<i>3.86</i>	<i>0.80</i>	<i>1.00</i>	<i>11.80</i>
Rotation----kg N ha <sup>-1</sup> ----					
Conventional tillage -CR	35.9 <sup>a</sup>	14.3 <sup>a</sup>	3.7 <sup>ab</sup>	4.9 <sup>a</sup>	58.7 <sup>a</sup>
Conventional tillage +CR	30.3 <sup>a</sup>	15.7 <sup>a</sup>	2.5 <sup>b</sup>	4.6 <sup>a</sup>	53.2 <sup>a</sup>
Reduced tillage -CR	37.7 <sup>a</sup>	14.8 <sup>a</sup>	4.1 <sup>ab</sup>	5.1 <sup>a</sup>	61.7 <sup>a</sup>
Reduced tillage +CR	52.0 <sup>a</sup>	19.5 <sup>a</sup>	5.1 <sup>a</sup>	5.8 <sup>a</sup>	82.4 <sup>a</sup>
<i>Average</i>	<i>39.0</i>	<i>16.1</i>	<i>3.9</i>	<i>5.1</i>	<i>64.0</i>
<i>SE</i>	<i>9.18</i>	<i>2.62</i>	<i>0.72</i>	<i>0.63</i>	<i>10.51</i>

CR= crop residue, SE=standard error; values in the same column and cropping system followed by the same letter are not significantly different at P<0.05

### 5.3.5 Nitrogen balance

Overall, the net nitrogen balances varied from -19 to +8 kg N ha<sup>-1</sup> when only harvest soybean grain was removed, and from -51 to -9 kg N ha<sup>-1</sup> when also maize grain was removed (Table 5.6). With only soybean grain removed, a positive N balance was observed in 'reduced tillage plus crop residue' treatments. Although the balances were all in the negative range when both soybean and maize grain were removed, the balances in the rotation system, for example, were better in 'reduced tillage plus crop residue' than in the other treatments (P<0.05). Also, balances with soybean and maize grain removed were better in reduced tillage (-10 to -33 kg N ha<sup>-1</sup>) than in conventional

# Effects of tillage and crop residue on soybean nitrogen fixation

tillage (-42 to -66 kg N ha<sup>-1</sup>), assuming two seasons for the intercrop. The differences in the balances are a result of both the %NdfA and the amount of harvested products.

Table 5.6. Nitrogen balance in soybean-maize intercropping and rotation systems in Nyabeda, western Kenya, March-August 2007 cropping season

<i>Treatment</i>	<i>Total N fixed</i>	<i>Total N removed in harvested grain</i>		<i>Net balance</i> <sup>§</sup>	<i>Net balance</i> <sup>#</sup>	<i>Net balance</i> <sup>δ</sup>
		Soybean	Maize			
	Intercrop ----kg N ha <sup>-1</sup> ----					
Conventional tillage -CR	30.7 <sup>a</sup>	38.3 <sup>a</sup>	23.1 <sup>a</sup>	-10.0 <sup>b</sup>	-25.5 <sup>b</sup>	-33.1 <sup>b</sup>
Conventional tillage +CR	26.3 <sup>a</sup>	28.3 <sup>a</sup>	18.0 <sup>a</sup>	-3.3 <sup>ab</sup>	-22.0 <sup>ab</sup>	-21.3 <sup>ab</sup>
Reduced tillage -CR	28.9 <sup>a</sup>	21.2 <sup>a</sup>	17.4 <sup>a</sup>	7.9 <sup>a</sup>	-6.2 <sup>a</sup>	-9.4 <sup>a</sup>
Reduced tillage +CR	47.5 <sup>a</sup>	43.5 <sup>a</sup>	18.2 <sup>a</sup>	3.4 <sup>ab</sup>	-19.9 <sup>ab</sup>	-14.9 <sup>ab</sup>
<i>Average</i>	<i>33.4</i>	<i>32.8</i>	<i>19.2</i>	<i>-0.5</i>	<i>-18.4</i>	<i>-19.7</i>
<i>SE</i>	<i>11.80</i>	<i>10.87</i>	<i>4.02</i>	<i>4.3</i>	<i>5.5</i>	<i>6.1</i>
	Rotation ----kg N ha <sup>-1</sup> ----					
Conventional tillage -CR	58.7 <sup>a</sup>	63.8 <sup>a</sup>	47.1 <sup>a</sup>	-4.3 <sup>ab</sup>	-27.2 <sup>a</sup>	-51.4 <sup>b</sup>
Conventional tillage +CR	53.2 <sup>a</sup>	72.7 <sup>a</sup>	25.3 <sup>b</sup>	-18.6 <sup>c</sup>	-48.6 <sup>b</sup>	-43.9 <sup>b</sup>
Reduced tillage -CR	61.7 <sup>a</sup>	72.5 <sup>a</sup>	21.4 <sup>b</sup>	-11.3 <sup>bc</sup>	-44.9 <sup>b</sup>	-32.7 <sup>b</sup>
Reduced tillage +CR	82.4 <sup>a</sup>	76.3 <sup>a</sup>	15.1 <sup>b</sup>	4.7 <sup>a</sup>	-26.8 <sup>a</sup>	-10.4 <sup>a</sup>
<i>Average</i>	<i>64.0</i>	<i>71.3</i>	<i>27.2</i>	<i>-7.4</i>	<i>-36.9</i>	<i>-34.6</i>
<i>SE</i>	<i>10.51</i>	<i>10.75</i>	<i>4.92</i>	<i>3.3</i>	<i>4.4</i>	<i>6.6</i>
<i>Tillage</i>			*			*
<i>Crop residue</i>			*			*
<i>Tillage x crop residue</i>				**	**	

<sup>§</sup> only soybean grain yield removed; <sup>#</sup> soybean grain, biomass and husks removed; <sup>δ</sup> soybean and maize grain removed; CR= crop residue; SE= standard error; values in the same column and cropping system followed by the same letter are not significantly different at P<0.05; \* significant effect at P<0.05; \*\* significant effect at P<0.01

## **5.4 Discussion**

### **5.4.1 Factors affecting nitrogen fixation**

The greater %NDfA observed in reduced tillage mainly when crop residue was also applied, and the resultant higher amount of fixed N compared to disturbed soils (such as in conventional tillage) has been attributed to the greater colonization by arbuscular mycorrhizae that increase P supply to the nodules (Goss and de Varennes 2002). We also observed higher fungal presence under reduced tillage compared to conventionally tilled plots (this study), similar to other studies (Beare et al. 1993; Degens et al. 1996). Legumes are generally dependent on mycorrhizae for efficient uptake of P, and diversity of mycorrhizae is related positively to efficient use of soil P (Vance 2001). Besides mycorrhizae, it was previously shown that Bradyrhizobia diversity is higher under reduced tillage, and N fixation rates of the isolates obtained from reduced tillage are also higher than in the case of conventional tillage (Ferreira et al. 2000). This could also apply to our reduced tillage treatments, and the diversity of rhizobia and fixation efficiency were likely greater with than without surface crop residue, leading to the greater nitrogen fixation in 'reduced tillage plus crop residue' relative to other treatments as observed. But during the first seasons, microbial communities were likely not yet well established, and available soil P differences between the tillage systems were likely more pronounced (crusting was a greater problem) than during season 9 when the  $^{15}\text{N}$  dilution method was employed. This explains the inconsistency between nodulation (higher in conventional tillage) and %NDfA (higher in reduced tillage) during the two time periods.

In addition to microbial contributions and soil P, reduced nodulation (and darker-green soybean leaves and visually observed tendency to delay maturation) following chemical fertilizer N application is due to the suppressive effect of the increased available N. Similar findings have also been reported by other researchers (Ralston and Imsande 1983; van Kessel and Hartley 2000). The reduced %NDfA and fixed N in conventional tillage as opposed to reduced tillage (e.g., in the rotation system) can also be attributed to the suppressive effect of a possibly larger amount of available soil N than in reduced tillage systems (Peoples and Craswell 1992; van Kessel and Hartley 2000; Wheatley et al. 1995). Such greater N in conventional as opposed to reduced tillage can be the result of the usually higher mineralization of nutrients in

organic matter following perturbations by tillage (van Kessel and Hartley 2000). With conventional tillage, the stronger depressive effect of incorporated crop residue on %NDfA in rotation as opposed to intercropping may be due to the fact that the mineralized N was fully at the disposal of the soybean alone, whereas this was shared among the jointly planted crops (soybean and maize) in the intercropping system. A similar effect was not observed with nodulation (greater nodulation in conventional than in reduced tillage), which was perhaps mainly influenced by differences in soil P as already discussed. Unlike with incorporated residue, the positive effect with surface residue (reduced tillage) on N fixation might be attributed to the role of residues in micro-climate modification, reduced plant water stress during the dry periods due to soil moisture conservation (leading to increased plant demand for fixed N (Streeter 2003)), and reduced soil temperature (Power et al. 1986); these factors were likely minimized or absent when surface crop residue was not applied or when much of it was incorporated into the soil (e.g., in conventional tillage).

Lower soybean %NDfA in the intercropping system is in agreement with previous reports such as the 42 and 23% NDfA found under monocrop and intercrop reported by van Kessel and Hartley (2000). Fujita et al. (1992) identified shading and light availability as important factors reducing BNF in mixed legume-cereal systems. Perhaps the lack of crop residue effect on %NDfA observed under the intercropping system was due to soybean-maize interaction effects such as shading and nutrient and water competition.

#### **5.4.2 Nitrogen fixed and N balances**

In general, fixed N in the intercropping system was just about 50% of the fixed N in the rotation system. However, assuming two cropping seasons in a year, and assuming that a similar amount of N is fixed during the second intercrop season during the cereal phase of the rotation system, similar quantities of N could be fixed annually in both intercropping and rotation. The amount of fixed N in the rotation system was within the range of 51 to 78 kg N ha<sup>-1</sup> observed by Osunde et al. (2003) in various farms in Nigeria. Other researchers have reported different amounts of fixed N. For instance, Peoples et al. (1995) reported N fixed by soybean of between 44 to 250 kg N ha<sup>-1</sup> per season. Our results imply that in both cropping systems, practicing reduced tillage while

adding crop residue results in the greatest amounts of biologically fixed N, which is in line with our hypothesis. The reported quantity of fixed N in the current study could be an underestimation, since an appreciable amount of fixed N in soybean, reaching up to 30% of the N being fixed at pod-filling (Ofosu-budu et al. 1990), is said to be released by the root system into the soil nutrient medium (Fujita et al. 1992).

Nitrogen balances of crop fields that include grain legumes vary widely and are affected by site conditions, grain harvest and N input (van Kessel and Hartley 2000). Although in the present study 42% to 65% of soybean N was obtained from BNF, its contribution to the overall N balance was small. Similar soybean N balances have been reported for Argentina (Di Ciocco et al. 2008) and Switzerland (Oberson et al. 2007). In fact, in their review of BNF studies that had been conducted between 1966 and 2006, Salvagiotti et al. (2008) observed that the amount of fixed N in soybean (a grain legume as opposed to non-grain legumes or where the grain is usually not harvested) was, in most cases, insufficient to replace all the N removed in harvested seed. Some positive N balances after soybean seed harvest were found in our study mainly in the ‘reduced tillage plus crop residue’ treatments, suggesting that adoption of ‘reduced tillage plus crop residue’ could save some cash the farmers would otherwise spend on fertilizers. For instance, for two cropping seasons (annually), an additional 10 to 30 kg N ha<sup>-1</sup> is required in reduced tillage, which is about half or less of the 40 to 60 kg N ha<sup>-1</sup> required in conventional tillage to compensate for harvested N. The farmers would also achieve a higher soybean grain yield over other treatments of 50 to 160 kg ha<sup>-1</sup> in rotation and 70 to 360 kg ha<sup>-1</sup> in intercropping by using ‘reduced tillage plus crop residue’. But although soybean can have a positive N balance (after soybean grain removal), the fixed N cannot also compensate for the entire N harvested in the maize grain. Chikowo et al. (2004) studied a similar case in a sandy soil in southern Africa and concluded that a combination of legume rotations and mineral fertilizer application would be the best option.

In terms of residue management, small-scale farmers usually transfer above-ground biomass to the homestead, where threshing is done with ease, and the residues are heaped in a corner where they rot. As such residues contain total N of up to 30 kg N ha<sup>-1</sup> such as for the rotation system, we recommend that soybean residues after removal

of grains be returned to the field plots after harvest, especially in the soybean-maize rotation system, to reduce soil N mining.

#### **5.4.3 Litter-fall- and root-N contribution**

Nitrogen contained in litter-fall occurring from planting to physiological maturity in this study was 8.2 to 11.8 kg N ha<sup>-1</sup>, but as much as 40 kg N ha<sup>-1</sup> can be observed in legume systems (Peoples and Craswell 1992). Our accounting took into consideration the 65% of litter-fall that had occurred before physiological maturity, constituting fixed N of 4 to 6 kg N ha<sup>-1</sup>, representing 54% NDfA. Nitrogen contained in such litter-fall is not usually taken into consideration in estimation of N-fixation nutrient balances, perhaps because of the small amount of the fixed N, and some of it can be lost within the season.

In both cropping systems, the recovered roots accounted for about 8% of the total plant biomass, consistent with the observation of Danso et al. (1993) that underground grain legume plant parts constitute <10% of total standing biomass. The roots may, however, not contribute substantially to fixed N, as they seem to be enriched in <sup>15</sup>N due to isotope <sup>15</sup>N/<sup>14</sup>N fractionation. The fractionation is related to form and concentration of N in the soil, nitrate reduction in roots, ammonia assimilation, and plant-fungal associations (Högberg 1997; Yoneyama et al. 2003). Wanek and Arndt (2002) reported  $\delta^{15}\text{N}$  enrichment of soybean roots at high %NDfA, but such enrichment was reversed to the shoots when soil nitrate was high, induced by the switch from nodule activity to mineral N acquisition. This may explain why roots, although not usually reported in many N fixation studies (van Kessel and Hartley 2000), are generally found to have more <sup>15</sup>N or  $\delta^{15}\text{N}$  than shoots (Ruschel et al. 1979; Steele et al. 1983).

In summary, ‘reduced tillage plus crop residue’ resulted in the highest %NDfA and total N fixed and better N-balances than ‘reduced tillage minus crop residue’ and conventional tillage treatments. Thus, in agreement to our hypothesis, practicing reduced tillage combined with crop residue application leads to greater utilization of BNF than the other treatments.

## 5.5 Conclusions

Small-scale farmers in western Kenya can take greater advantage of biological N fixation by (i) practicing reduced tillage combined with surface application of crop residue, as this increases biologically fixed N by at least 34% over other treatments tested, (ii) applying chemical fertilizer phosphorus, and (iii) returning soybean residues to the farm after grain removal, as such residues contained total N as much as 30 kg N ha<sup>-1</sup>. By practicing 'reduced tillage plus crop residue', farmers can achieve extra soybean grain yields of 50 to 160 kg ha<sup>-1</sup> in rotation and 70 to 360 kg ha<sup>-1</sup> in intercropping, and better N balances can be realized than when practicing conventional tillage. Soybean roots get enriched with <sup>15</sup>N due to isotope <sup>15</sup>N/<sup>14</sup>N fractionation and do not contribute substantially to total plant fixed N.

## **6 EFFECT OF TILLAGE, CROP RESIDUE AND MINERAL FERTILIZER APPLICATION ON MAIZE AND SOYBEAN PRODUCTIVITY**

### **6.1 Introduction**

Conservation tillage offers an opportunity to reverse the land degradation that prevails in many parts of sub-Saharan Africa (Fowler and Rockstrom 2001) due to its positive effects on enhancement of soil physical, biological and chemical properties when compared to conventional tillage practices (Madari et al. 2005; Wander and Yang 2000). However, in Kenya, as in many other countries in sub-Saharan Africa, conservation tillage has not been widely adopted (Rockstrom et al. 2003), and the majority of farmers still practice seasonal and mid-season tillage operations. Among the reasons for low adoption of conservation tillage by smallholder farmers in eastern Africa is the low crop productivity under conservation tillage compared to the conventional tillage systems commonly practiced by farmers (Taa et al. 2004). Positive effects on crop productivity resulting from the effects of improved soil-plant moisture and nutrient relations in reduced tillage generally become evident only after long periods of applying the technique (Ghuman and Sur 2001; Malhi et al. 2006). However, such long-term evaluation is hampered by the lack of trials with sufficient replication in time. In Kenya, conservation tillage studies have traditionally concentrated on the drier zones where soil moisture is often limited and soil erosion is prevalent (Biamah 2005), with little work in the sub-humid zone. A long-term experiment involving reduced tillage was therefore established in the sub-humid zone in 2003 in western Kenya to evaluate crop performance under the reduced tillage compared to the conventional tillage system.

Crop residues are an important component of reduced tillage and affect crop yields by reducing runoff and direct evaporation from the soil (Biamah 2005), besides also adding nutrients to the soil (Erenstein 2003). Crop residue effects on yields vary, for example, according to the crop residue type, quantity (Scopel et al. 1998), placement and the rate of its disappearance (Erenstein 2003). Finding sufficient quantities of crop residue for use as mulch is often a problem in the smallholder farms in Africa, due to



competing uses such as for fodder and fuelwood (Fowler and Rockstrom 2001). Many studies, however, use unrealistic quantities of crop residue (Kimani et al. 2007; Mtambanengwe et al. 2007), and the results can then not be easily transferred to the on-farm situation. If crop residue is to be tested under conservation tillage, it is best to use amounts that the smallholder farmers realistically have access to. Fowler and Rockstrom (2001) implied such accessible crop residue amounts to be 2 to 3 t ha<sup>-1</sup>, and the amount of maize residues that can provide the 30% soil cover required in reduced tillage systems at planting is estimated at about 2 t ha<sup>-1</sup> (Erenstein 2003).

Crop rotation, such as the planting of legumes after cereal crops is one of the principles of conservation tillage (Benites 2008; Erenstein et al. 2008). However, in Kenya, besides continuous cereal monocropping and legume-cereal rotation, some farmers also practice legume/cereal intercropping. In either rotation or intercropping, legumes can minimize crusting problems that are sometimes associated with low yields in reduced tillage relative to conventional tillage systems (Hoogmoed 1999), and this can be reflected in yields. In Kenya, it is not clear to what extent having a legume in the cropping system can affect agronomic performance and profitability of reduced tillage relative to the conventional tillage system, compared to where legumes are not included. Testing the different tillage systems in different cropping systems that represent farmers' practices offers the best way to enable comparisons of their performance. The aim of this study was to identify those cropping systems used by small-scale farmers in which reduced tillage practices that avoid the known negative impacts of continuous tillage might achieve similar or higher yields compared to the conventional tillage practices.

This study investigates the effect of tillage and crop residue application on maize and soybean productivity over several seasons in different cropping systems on the predominant clay soil in western Kenya. We hypothesized that;

- (i) Crop yield following a modest application of  $2 \text{ t ha}^{-1}$  of crop residue in a reduced tillage system is similar to the yield obtained from a conventional tillage system, and that
- (ii) Incorporation of legumes in a cropping system, as rotation and also as intercrop, leads to greater economic benefits as opposed to a cropping system involving continuous maize.

## **6.2 Materials and methods**

### **6.2.1 Study location**

The study was conducted in an on-farm researcher-managed experiment in Nyabeda, western Kenya. The mean annual rainfall is 1580 mm and is distributed in two rainy seasons: a long rainy season from March to August (about 900 mm) and a short rainy season from September to February (about 680 mm; see also section 1.5).

### **6.2.2 Experimental design and treatments**

The experiment had been set up in 2003 as a split-split-split plot design with 4 replicates and involved a factorial combination of tillage system (reduced and conventional tillage), cropping system (continuous cereal, soybean-maize rotation and intercropping), crop residue - maize stover - management (plus and minus crop residue) and nitrogen (N) application (12 treatments; see Table 1.2). For continuous maize, treatments were split to accommodate an N response, using 0, 30, 60 and  $90 \text{ kg N ha}^{-1}$ . Rotation plots were split into four to accommodate 0 and  $60 \text{ kg N ha}^{-1}$  each for legume and cereal crops. Since all treatments received phosphorus (P), additional rotation treatments without P were incorporated to enable investigation of P effects. All intercropping treatments did not receive N but only  $60 \text{ kg P ha}^{-1}$ .

Maize was planted at 0.25 m (between plants) by 0.75 m (between rows) with two seeds per planting hole and thinned to one plant per hill ( $53,000 \text{ hills ha}^{-1}$ ). Soybean was planted at 0.05 m by 0.75 m ( $266,000 \text{ seeds ha}^{-1}$ ). Maize was harvested from the

plots at maturity leaving 2 border plants (0.25 m spacing) on both ends of the row and one row (0.75 m spacing) from the other ends to eliminate edge effects. Cobs were separated from the stover and the fresh weight of each determined. After air drying the sub-sample, grains were separated from the cobs, oven-dried at 60°C for 48 hours and their separate dry weights determined. Similarly, legume grain and biomass, harvested from equal plots as maize, were measured on an oven-dry basis.

Fertilizer K (from murate of potash) was applied to all plots at 60 kg ha<sup>-1</sup> while P (from triple super phosphate -TSP) and N (from urea) were applied at the specified rates every season. N was split-applied with 1/3 at planting and 2/3 at knee height (5 weeks after planting). Fertilizer application method was hill placement (see also section 1.5).

### **6.2.3 Determining economic benefits**

Partial budget analysis was carried out following the methodology of CIMMYT (1988). Cumulative input and output data for the first 9 seasons (except the drought season i.e., season 6 (see section 6.3.1, *Seasonal trend of maize yield*)) were used. For the partial budgeting, the treatments in the continuous cereal system had 60 kg N ha<sup>-1</sup> applied, while those in intercropping and rotation had no N applied. To reflect the difference between experimental yield and the yield that farmers could expect, the yields were adjusted downwards for farmer management by 10% and a further 5% for small plot size (Spencer 1993). Farm gate price of output at harvest (using the prices of the year 2007) was US\$ 16.7 [1 US\$ to 72 Kenya Shillings] for a 90 kg bag of maize and US\$ 62.5 for a 90 kg bag of soybean. Since maize stover is often used as a source of fuelwood and livestock feed in the study area, opportunity cost was used to determine its imputed (derived) price at US\$ 13.9 per tonne. Gross revenue was calculated by multiplying yield data with the relevant constant price. The price of maize and soybean seed planted was US\$ 0.69 per kg. Farm gate cost of fertilizer was US\$ 22.2 per 50 kg bag of urea and US\$ 25.7 per 50 kg bag of TSP. These, together with costs of labor to plant and weed were used to calculate the total variable costs. Gross margin was calculated as the difference between gross revenue and total variable costs. Sensitivity analysis was done by including plus and minus one standard error for each of the practices tested, in order to reflect the range of gross margins that can be expected for

each practice based on seasonal yield variability. A similar sensitivity analysis approach has been used before (Tursunov 2009).

Dominance analysis was done to determine those practices with lower gross margins (and higher total costs that vary) than other practices with higher gross margins (and lower total variable costs); the former practices are usually then considered dominated by the latter. Since the dominated options are not the best to be recommended to farmers, they were usually eliminated from further consideration (e.g., in the calculation of marginal rate of returns (MRR) needed to further fine-tune farmer recommendations) in order to focus attention on the non-dominated alternatives. Marginal rate of returns were calculated as the ratio of the difference between the additional benefit gained and the additional cost incurred from a switch from one non-dominated option to another.

#### **6.2.4 Data analysis and presentation**

The data were analyzed using Statistical Analysis Software (SAS) version 9.1 for Windows. The mixed model procedure was used for analysis of variance between treatments allowing for analysis of random effects of season, replicate, tillage practice and applied crop residue. Multiple pair-wise comparisons of least square means were done directly in SAS using the Tukey-Kramer method.

### **6.3 Results**

#### **6.3.1 Seasonal trend of maize yield**

Seasonal maize grain yield varied considerably as expected, mainly influenced by rainfall amount and distribution (Figure 6.1). For example, the extremely low yields in season 6, which had similar rainfall as season 2, were attributed to severe crop water-stress, as only <60 mm rainfall was received after 70 days after planting in that season. The last three seasons (seasons 7-9) coincidentally had greater seasonal rainfall than the earlier seasons, but this did not translate to greater maize yields. For example, the yield in season 7 was likely reduced due to excess soil water in the clay soil, as up to 500 mm rainfall was received by only 35 days after planting.

Except for the drought season (season 6) and season 1, yield in the conventional tillage system was slightly to considerably more (by 6% to 25%) than in

reduced tillage, perhaps resulting from increased nutrient mineralization and hence, plant uptake when the soil was tilled. Since season 6 resulted in crop failure, it was excluded from the subsequent analysis involving averaging the yields over the March 2003 to August 2007 period. Also, for the legume analysis season 1 was excluded, as a different legume (common beans) had been grown.

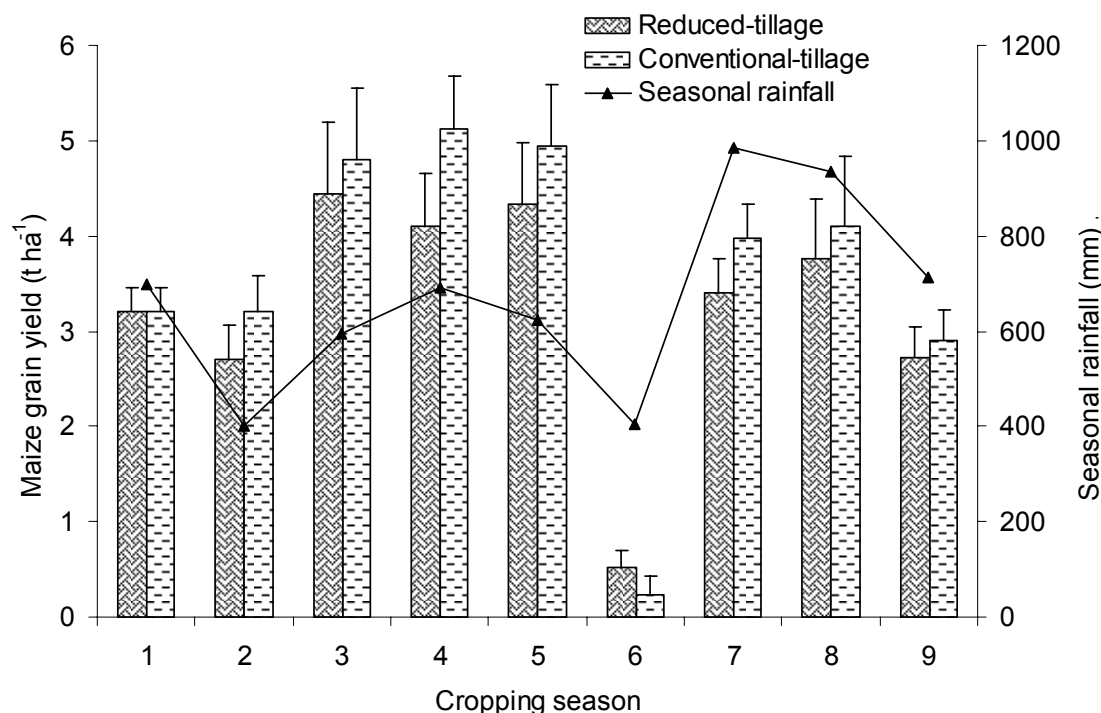


Figure 6.1. Maize grain yield in reduced and conventional tillage as observed in continuous maize cropping system in Nyabeda western Kenya, March 2003 to August 2007. Nitrogen and phosphorus fertilizers at 60 kg ha<sup>-1</sup> and 2 t ha<sup>-1</sup> of crop residue were applied every season in both treatments; error bars are standard errors

### 6.3.2 Effect of N and P fertilizers on maize yield

For both reduced and conventional tillage systems, the effects of N and P fertilization were first investigated to determine the best options for further analysis. As expected, maize responded to fertilizer N in both reduced and conventional tillage systems (Figure 6.2). Applying 30 kg N ha<sup>-1</sup> increased yield by about 40% ( $P < 0.05$ ) over the control (zero N application) in both tillage systems and a further 26% ( $P < 0.05$ ) and 15% (not significant) in the reduced and conventional tillage systems, respectively, at 60 kg N ha<sup>-1</sup>. The response to N application in this continuous maize cropping system beyond 60 kg N ha<sup>-1</sup> was small (13% and 2% in reduced and conventional tillage, respectively).

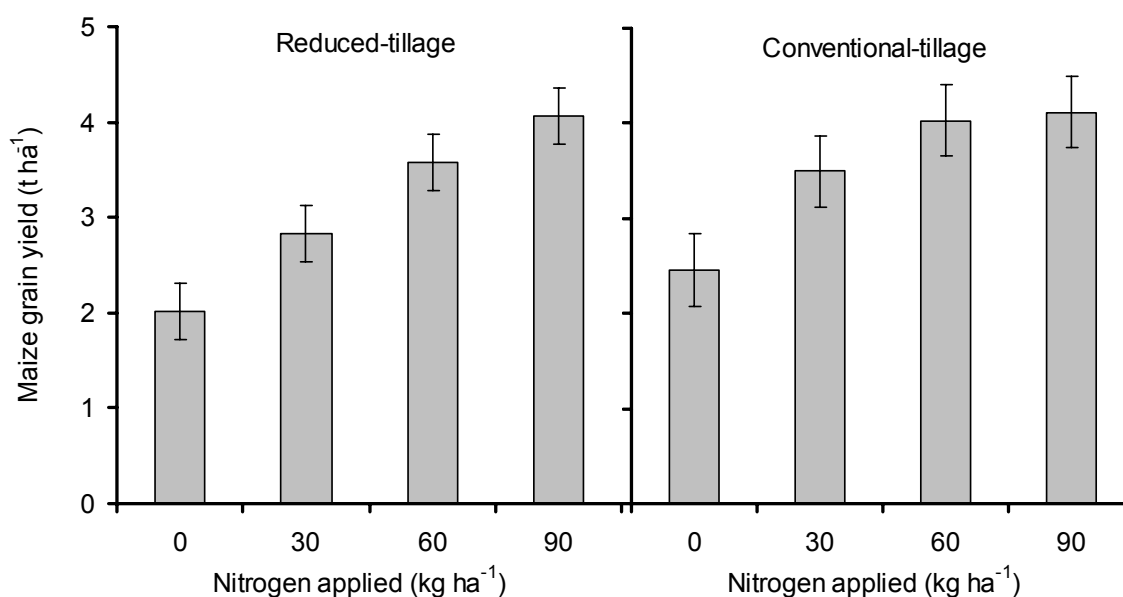


Figure 6.2. Effect of N fertilization on maize grain yield in continuous maize cropping system under two tillage practices in Nyabeda, western Kenya, March 2003 to August 2007. Bars represent LSD comparing means within the specific tillage system; All treatments had crop residue applied at 2 t ha<sup>-1</sup>; season 6 (crop failure) not included; P was applied to all treatments at 60 kg P ha<sup>-1</sup>.

Combined application of N and P fertilizers (+P+N) resulted in higher maize grain yields in conventional tillage (25%) and reduced tillage (54%) than for P applied alone (+P-N; Figure 6.3). Also, applying P alone was better than no P at all, increasing the grain yield by 100% in conventional tillage and 80% in reduced tillage over the yield in the no P control treatments (-P-N). The maize yield in the rotation system for the '+P-N' regime was somewhat similar to that in continuous cereal at 60 kg N ha<sup>-1</sup> (also applied with P; see Figure 6.2), for both reduced and conventional tillage, indicating that the maize benefited from residual effects of the legume. Therefore, only the '+P-N' treatments in rotation (and also in intercrop) and 60 kg N ha<sup>-1</sup> treatments in the continuous maize cropping system were investigated further in this study.

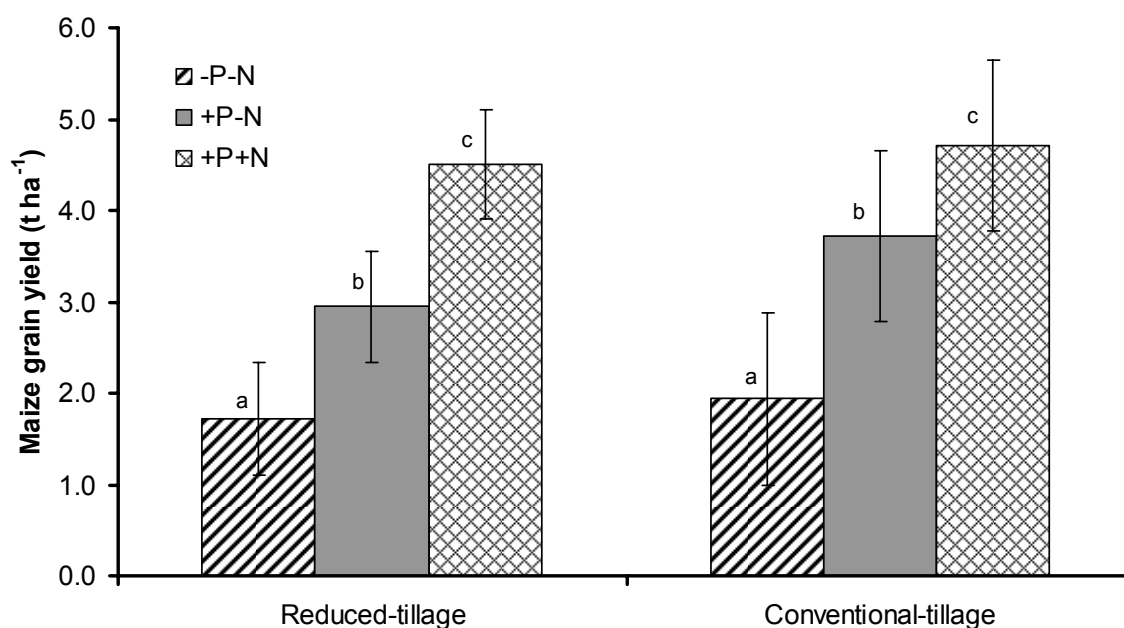


Figure 6.3. Effect of phosphorus and nitrogen fertilization on maize yield in soybean-maize rotation system in Nyabeda, western Kenya, March 2003 to August 2007; bars are confidence limits; different letters indicate significant differences; season 6 (crop failure) not included

### 6.3.3 Effects of tillage and crop residue

Seasonal average maize grain yields, over the March 2003 to August 2007 period, were 3.2-4.1 t ha<sup>-1</sup> in continuous maize, 3.0-3.9 t ha<sup>-1</sup> in soybean-maize rotation and 1.8-2.8 t ha<sup>-1</sup> in the soybean-maize intercropping system (Table 6.1). The lower yield in the intercropping system was expected due to competition for soil water and nutrients with the accompanying legume.

Practicing conventional tillage resulted in 11-26%, 17-30% and 36-58% higher grain yields than reduced tillage, in continuous maize, soybean-maize rotation and intercropping systems, respectively. Lower yield with reduced tillage was attributed to soil surface crusting, resulting in surface runoff, nutrient losses and reduced infiltration, and hence greater plant-water stress than in the conventional tillage system. The intercropped maize likely suffered a higher water and nutrient stress due to the ensuing competition, hence the greater reduction in yield in intercrop relative to rotation and continuous maize. Stover yield followed a pattern somewhat similar to that of grain yield, having 4-24%, 12-26% and 14-35% higher yields in conventional than in reduced tillage in continuous maize, rotation and intercropping systems, respectively. We did not

find yield differences due to tillage in continuous maize and rotation systems during the last 3 seasons (seasons 7-9), although there were differences in the previous seasons (seasons 2-5; see Appendix 2). Although those last three seasons received higher rainfall than the earlier seasons as already shown, the rainfall distribution in season 9 was poor (only 70 mm rainfall received between 58 and 106 days after planting), and thus the lack of differences in yield during the last seasons is likely due to soil improvement and not just higher rainfall.

There was no significant effect of crop residue on yield and only small variations (-9% to +11%) were observed. Also, the tillage x crop residue interaction effect observed for maize stover yield in continuous maize resulted in only an 8% yield increase in reduced tillage and an 8% yield decrease in conventional tillage, when residue was added. The decrease in yield following incorporation of low-quality crop residue in conventional tillage is usually associated with immobilization of soil available N, while surface application results in benefits such as soil-water conservation. The decrease in yield in the conventional tillage system following application of crop residue was greater during the first 5 seasons, but additive yields over the no-crop residue treatments were observed in some cases during the last 3 seasons (Figure 6.4). Thus, application of crop residue reduced yield to a greater extent during the earlier seasons than during the last seasons, perhaps due to slow improvement in soil fertility with continued fertilization. This pattern was only observed in treatments that had not received chemical fertilizer nitrogen, implying that the applied crop residue led to immobilization of some of the available soil nitrogen.



Table 6.1. Maize grain and stover yield ( $\text{t ha}^{-1}$ ) in continuous maize, soybean-maize rotation and soybean/maize intercropping in Nyabeda, western Kenya, March 2003 and August 2007

<i>Treatment</i>	<i>Continuous Maize</i>		<i>Rotation</i>		<i>Intercrop</i>	
	Grain	Stover	Grain	Stover	Grain	Stover
	--- $\text{t ha}^{-1}$ ---					
Reduced tillage -CR	3.24 <sup>c</sup>	3.66 <sup>b</sup>	3.18 <sup>b</sup>	3.55 <sup>bc</sup>	1.75 <sup>b</sup>	2.53 <sup>bc</sup>
Reduced tillage +CR	3.58 <sup>bc</sup>	3.98 <sup>b</sup>	3.00 <sup>b</sup>	3.27 <sup>c</sup>	1.89 <sup>b</sup>	2.36 <sup>c</sup>
Conventional tillage -CR	3.97 <sup>ab</sup>	4.54 <sup>a</sup>	3.91 <sup>a</sup>	4.11 <sup>a</sup>	2.77 <sup>a</sup>	3.18 <sup>a</sup>
Conventional tillage +CR	4.07 <sup>a</sup>	4.14 <sup>ab</sup>	3.74 <sup>a</sup>	3.96 <sup>ab</sup>	2.58 <sup>a</sup>	2.89 <sup>ab</sup>
<i>SED</i>	0.20	0.24	0.20	0.25	0.16	0.20
<i>Tillage</i>	**	**	**	**	**	**
<i>Tillage x crop residue</i>	-	*	-	-	-	-

Numbers in the same column followed by a different letter are significantly different at  $P < 0.05$ ; CR= crop residue; SED=standard error of the differences of means; \*significant at  $P < 0.05$ ; \*\*significant at  $P < 0.01$ ; crop failure season (season 6) not included

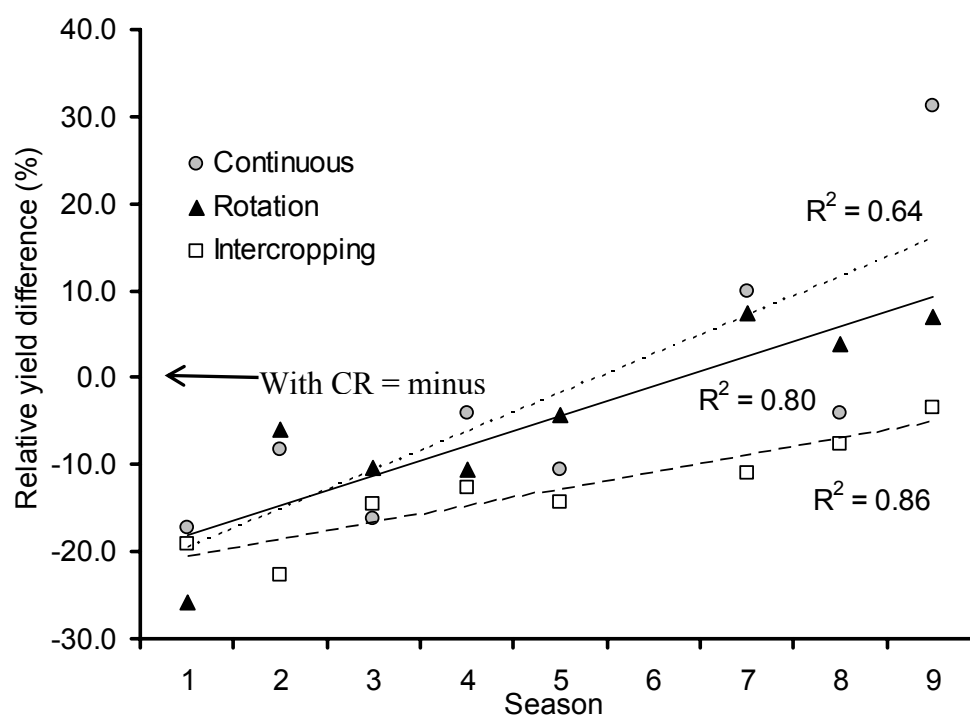


Figure 6.4. Trend of relative differences in maize grain yield between treatments with and without crop residue in different cropping systems under conventional tillage in Nyabeda, western Kenya, March 2003 to August 2007. Drought season (season 6) not included; all treatments received  $60 \text{ kg P ha}^{-1}$  but not N; CR= crop residue

Seasonal average soybean grain yields were from 0.92-0.99 t ha<sup>-1</sup> in the soybean-maize rotation and from 0.52-0.60 t ha<sup>-1</sup> in the soybean/maize intercropping system (Table 6.2). Although the soybean grain and aboveground biomass yields were expected to be affected by tillage and crop residue similarly to maize, no such effects were observed. The lack of differences can be attributed to quick establishment and maximum canopy (reaching up to 100% in about 2 months after planting), which completely covered the soil and protected soil water from surface evaporation. The soil under the bushy soybean was visually observed to be wetter than in the other cropping systems.

Table 6.2. Average soybean grain and aboveground biomass yield (t ha<sup>-1</sup>) in soybean-maize rotation and intercropping systems in Nyabeda, western Kenya, March 2003 and August 2007.

<i>Tillage</i>	<i>Crop residue</i>	<i>Rotation</i>		<i>Intercrop</i>	
		Grain	Biomass	Grain	Biomass
		---t ha <sup>-1</sup> ---		---t ha <sup>-1</sup> ---	
Reduced tillage	-CR	0.95	1.66	0.56	1.01
Reduced tillage	+CR	0.92	1.66	0.60	1.15
Conventional tillage	-CR	0.99	1.75	0.52	1.01
Conventional tillage	+CR	0.98	1.72	0.53	1.07
<i>SE</i>		<i>0.107</i>	<i>0.144</i>	<i>0.092</i>	<i>0.158</i>

CR=crop residue; all treatments received 60 kg P ha<sup>-1</sup> but not N; season 1 (common beans) and crop failure season (season 6) not included; SE=standard error

#### 6.3.4 Partial budget analysis and marginal rate of return (MRR)

For each of the tillage practices and crop residue systems, the gross margins over the nine seasons were positive and ranged from US\$ 247 in the continuous cereal ('reduced tillage minus crop residue') to US\$ 435 in the soybean-maize intercropping system ('conventional tillage minus crop residue') (Table 6.3). These gross margins were influenced by the cropping system and were in the order intercropping >rotation >continuous maize.

All the treatments in continuous maize cropping systems were dominated, having 5% to 28% lower gross margins than those of corresponding legume-based treatments (rotation and intercropping), the latter having 3% to 33% lower total variable

costs. The other dominated options are intercropping treatments (except ‘conventional tillage minus crop residue’) and soybean-maize rotation treatments that had received crop residue. Among the non-dominated alternatives, the gross margins were in the order ‘conventional tillage minus crop residue’ in intercropping > ‘conventional tillage minus crop residue’ in rotation > ‘reduced tillage minus crop residue’ in rotation. The marginal rate of return (MRR) computed for the three non-dominated alternatives showed that by adopting reduced tillage farmers would lose revenue, since conventional tillage had a MRR of at least 182% over reduced tillage (Figure 6.5). This is due to the fact that the saving in labor under reduced tillage was not sufficient to compensate for the lower income from the lower yield obtained under this practice. A change from ‘conventional tillage minus crop residue’ in soybean-maize rotation to ‘conventional tillage minus crop residue’ in soybean-maize intercropping was not very appealing, as the MRR was only 48%.

Table 6.3. Average seasonal gross margins (US \$ ha<sup>-1</sup>) of different tillage and crop residue combinations in different cropping systems in Nyabeda, western Kenya, March 2003 to August 2007

Kenya, March 2005 to August 2007								
No.	Treatment	Cropping system	Gross revenue		TVC		Gross margin	
			Mean	SE	Mean	SE	Mean	SE
---US \$ ha <sup>-1</sup> ---								
1	Reduced tillage -CR	CM	553	182	306	17	247	165
2	Reduced tillage +CR	CM	611	115	321	10	289	105
3	Conventional tillage -CR	CM	679	157	356	14	322	143
4	Conventional tillage +CR	CM	690	151	368	14	322	137
5	Reduced tillage -CR	Intercrop	636	273	292	20	344	254
6	Reduced tillage +CR	Intercrop	680	338	305	24	374	313
7*	Conventional tillage -CR	Intercrop	781	424	345	31	435	393
8	Conventional tillage +CR	Intercrop	753	375	352	27	401	347
9*	Reduced tillage -CR	Rotation	552	234	214	17	337	216
10	Reduced tillage +CR	Rotation	527	228	222	17	305	211
11*	Conventional tillage -CR	Rotation	624	184	240	13	384	171
12	Conventional tillage +CR	Rotation	607	209	248	15	359	195

CM=continuous maize; CR= crop residue; SE= standard error; \*= non-dominated alternatives; TVC=total variable costs

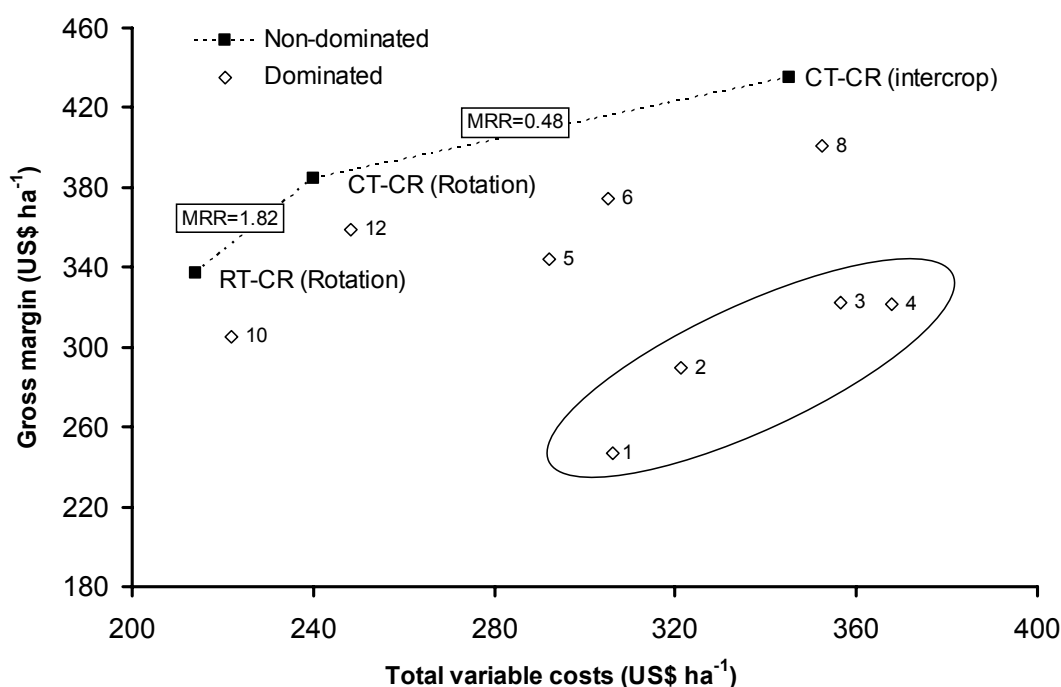


Figure 6.5. Average seasonal gross margins for different cropping systems, tillage and crop residue combinations in Nyabeda, western Kenya, March 2003 to August 2007. Numbers in the dominated options correspond to the treatment numbers in Table 6.3; RT= reduced tillage; CT= conventional tillage; CR=crop residue; MRR= marginal rate of return; circled treatments are continuous maize treatments tested; drought season (season 6) not included

## 6.4 Discussion

### 6.4.1 Tillage

Worldwide, research on agronomic performance of conservation tillage practices has generated contrasting results. For instance, lower yields in reduced tillage compared to conventional tillage practices have often been reported (Hoogmoed 1999; Laryea et al. 1991; Mazzoncini et al. 2008; Taa et al. 2004; Wilhelm and Wortmann 2004), but also higher yields have been observed (Ozpinar and Cay 2005; Six et al. 2002). The similar soybean yields under reduced and conventional tillage in our experiment suggest that soybean could be an excellent crop under reduced tillage conditions. Since yields were similar, soybean production in western Kenya is better done under reduced tillage due to the additional environmental benefits, such as greater biodiversity, better soil structure and reduced erosion (see also Landers 2008).

Lower cereal yields under reduced tillage than conventional tillage systems have been attributed to factors such as invasive grass species and higher competition by weeds (Bàrberi and Cascio 2001), making it necessary to reduce weeds through spraying with herbicides. Other factors are decreased N mineralization (Camara et al. 2003), surface crusting and soil compaction leading to low water infiltration, higher soil bulk density, high root penetration resistance and reduced porosity (Osunbitan et al. 2005; Rosolem et al. 2002) in reduced tillage as opposed to conventional tillage practices. Lower maize grain yield in reduced tillage observed in our study could be due to surface crusting, which was visually observed mainly during early crop growth when crop cover was also minimal. Increased runoff due to surface crusting in reduced tillage has been associated with P losses (Andraski et al. 2003), which limits crop growth. This was likely a key contributor to the low yields in reduced tillage in our study, as available soil P in the reduced tillage was in general lower than in conventional tillage (Appendix 1). Previously, it has been reported that the initially lower yields in reduced tillage increase after some seasons of continued practice (Ghuman and Sur 2001; Malhi et al. 2006). This is consistent with the results of our study, and also soil was better aggregated and microbial activity greater in the reduced than in the conventional tillage system during season 9 (this study). Thus, similar maize yields between reduced and conventional tillage systems can be expected within the 3<sup>rd</sup> year for western Kenya, although it may take more than 5 years for the intercropping system.

The greater reduction in yield in intercropping than in rotation and continuous maize in reduced tillage relative to conventional tillage was attributed to greater water stress in the intercropping than in the other systems. This is consistent with stronger responses to rainfall in the intercropping than in the other cropping systems (data not shown), which indicates increased demand for water resulting from competition among the intercropped crop components. Yamoah et al. (2003) also found intercropping millet with cowpea to lead to a higher soil moisture stress than in monocropped millet. The rainfall up to 100 days after planting in our study was better related with yield than was whole-season rainfall, and thus measures that improve plant and soil-water relations up to 100 days after planting are urgently needed especially, in the intercropping system.

#### **6.4.2 Effect of crop residue**

Several studies have reported positive effects of application of crop residue on yield (Doran et al. 1983; Erenstein 2003; Lal 1974). Our results show only slight variations in yields due to crop residue application. The low response to crop residue is in agreement with the finding of Erenstein (2003) that there are no clear immediate benefits of crop residue in sub-humid environments. Nevertheless, crop residue was earlier shown to significantly improve soil structure and N fixation in both reduced and conventional tillage systems (this study), demonstrating the need for seasonal application of crop residue in western Kenya, especially in the reduced tillage system. Also, the positive trends following continued application of crop residue as observed in our study indicate that farmers continuing with conventional tillage can apply crop residue and expect yield increases later. We propose that crop residue, even under conventional tillage, be preferentially surface-applied to increase its soil-water conservation effect and reduce the negative effects on yield.

Combining reduced tillage with surface residue has been shown to improve crop performance (Dam et al. 2005; Woyesa and Bennie 2004). The lower yield in ‘reduced tillage with crop residue’ than in the conventional tillage system in our study, especially during the first 5 seasons, indicates that the 2 t ha<sup>-1</sup> crop residue applied in our case may be insufficient to immediately boost productivity to match that of conventional tillage for all systems tested. A fast disappearance of crop residue of up to 50% in the first month was observed in this site (Chapter 4) and could result in a smaller contribution of the crop residue to the minimization of surface crusting and its associated problems. Thus, our hypothesis of similar or higher yield in ‘reduced tillage plus 2 t ha<sup>-1</sup> crop residue’ relative to conventional tillage is supported after more than three years of continued practice in continuous maize and soybean-maize rotation, but it takes longer for the intercropping system. We suggest that further investigations on the use of crop residue, based on the amount that farmers are most likely to access, explore supplementing the crop residue with additional soil cover resources such as use of cover crops.

#### **6.4.3 N and P fertilization**

Crop productivity in most parts of Africa is mainly limited by the availability of nitrogen and phosphorus (Schlecht et al. 2007), and this is also the case for western Kenya. Low use of fertilizer, estimated at only 8 kg ha<sup>-1</sup> in year 2002 in sub-Saharan Africa (Maatman et al. 2008; Morris et al. 2007) and less than 20 kg N ha<sup>-1</sup> for western Kenya (Okalebo et al. 2007), results in low yields, which are often less than 1.5 t ha<sup>-1</sup> for maize grain (Mugwe et al. 2009; Tittonell et al. 2008). The positive yield increase following application of fertilizer N and P is not unique to our study, as this has been reported in several other studies (Kamara et al. 2008; Kihara et al. 2008; Muleba 1999). The integration of legumes in the cropping system, as in the intercropping and rotation systems, results in positive improvements in subsequent cereal yields, believed to be due to legume N fixation benefits especially where all legume biomass is retained on the field, among other advantages (Kamara et al. 2008).

Yield increase due to P application is often high in soils with low available P (Gachengo et al. 1999), such as those of western Kenya where less than 4.0 mg P kg<sup>-1</sup> soil on smallholder farms is commonly observed (Tittonell et al. 2008). This shows that there is a need not only to use fertilizers but also to raise the levels applied. This requires that the cost of chemical inorganic fertilizer, which is frequently high in Africa (Kelly et al. 2003), 2 to 6 fold higher than that in Europe, North America and Asia in 2002 (Sanchez 2002) be addressed, since it is a major hindrance to fertilizer application especially among smallholder farmers.

#### **6.4.4 Economic analyses**

To improve farmers' incomes, it is important to pay more attention to net benefits than mere yields. Reduced cost of production such as reduced labor, fuel and machinery is a key advantage of reduced tillage practices (Fowler and Rockstrom 2001) and is reported to result in more net benefits in reduced than in conventional tillage (Knowler and Bradshaw 2006). For example, Astatke et al. (2003) found a higher gross margin of US \$ 132 per ha<sup>-1</sup> for wheat production in reduced than in conventional tillage in Ethiopia. In another study carried out over a 16-year period in Spain, no significant differences in gross margins between reduced and conventional tillage were found, although the margins tended to be higher for the former than for the latter tillage practice (Sánchez-

Girón et al. 2004). In contrast, economic assessment of a shorter-term (4 year) study in a tropical sub-humid environment similar to our site found that the conventional tillage with residue removed had the highest net benefits among the tillage and crop residue alternatives tested (Fischer et al. 2002). The savings in labor due to reduced tillage could not sufficiently offset the benefits of increased yields in conventional tillage system in our case. However, with longer periods of assessment, the economic benefits could increase in reduced tillage, since the yields for the last seasons in this study were similar among the tillage systems (see Appendix 2).

Legume-based systems are economically more attractive to farmers than continuous maize systems, as all the treatments in the latter were dominated. This is in agreement with Sánchez-Girón et al. (2004), who reported 16% higher gross margins in a wheat-vetch rotation compared to winter barley monoculture. Farmers should thus be advised to integrate legumes in their cereal production systems, either in rotation or in intercropping, for greater economic returns. The finding of highest net benefits in intercropping is consistent with the higher land equivalence ratios usually reported relative to monocropping systems (Bationo 2008; Niringiye et al. 2005; Worku 2004). However, farmers may still practice rotation, since the MRR gained by switching from conventional rotation to intercropping is not so attractive, being at the lowest minimum MRR of 50% generally acceptable to farmers (CIMMYT 1988).

## **6.5 Conclusions**

Soybean yields in western Kenya are similar in reduced and conventional tillage systems (i.e., not showing the typical crop yield reduction when introducing reduced tillage), and thus soybean is an excellent crop for reduced tillage systems. However, practicing conventional tillage results in 11% to 58% higher maize grain yield than reduced tillage, due to problems associated with surface crusting in the reduced tillage system. Our hypothesis of similar yields between ‘reduced tillage with crop residue’ (applied at 2 t ha<sup>-1</sup>) and the conventional tillage system treatments is supported only after three years of continued reduced tillage practice. Applying chemical fertilizer P increases maize grain yield by at least 80% compared to where P is not applied, and a further minimum of 25% when also N is applied. Continuous maize systems have 5% to 28% lower gross margins and 3% to 33% higher total variable costs than legume-based



cropping systems, and thus the legume-based systems are economically more attractive to farmers. More work is needed to supply greater soil cover in the reduced tillage system and avoid soil crusting problems, which lead to lower maize yields soon after reduced tillage is adopted.

## **7 GENERAL DISCUSSION, SUMMARY AND RECOMMENDATIONS**

### **7.1 General discussion**

Reducing soil disturbance by tillage is vital for sustainable agricultural productivity and is central to one of the main challenges of the 21<sup>st</sup> century, i.e., climate change (Birkas et al. 2008). ‘Plow deep and you will have corn to sell and to keep’ was a popular saying among farming communities some decades ago (Triplett and Dick 2008). Since the publication of the book “*Plowman’s folly*” (Faulkner 1943) that advocated for reduced tillage as opposed to continuous cultivation, reduced tillage has continued to gain acceptance in many parts of the world. Today, ‘farmers are...parking their plows’ to save the soil (Huggins and Reganold 2008). Unfortunately, much of the effort over the years has been focused on large-scale mechanized systems and rarely on the small-scale farming systems (Derpsch 2008) that characterize farming in Africa, where conventional tillage systems are still widely practiced. We employed new and old techniques to investigate the effects of tillage, crop residue and cropping systems on soil structure, microbial diversity, residue disappearance and termite activity, N fixation and crop yields in small-scale farmer systems in Kenya.

#### **7.1.1 Tillage system**

The future of agriculture lies in the various conservation tillage practices that minimize soil tillage, because soil structure, organic matter and soil organisms among other factors are improved when compared to conventional tillage systems (Nakamoto et al. 2006; Pikul et al. 2009). Key advantages of reduced soil disturbance are better soil aggregation, different community compositions of soil micro-organisms and higher proportion of nitrogen derived from the atmosphere (%NDfA; Chapters 2, 3 and 5, respectively) than under conventional tillage. Based on whole-soil genome studied using modern cutting-edge PCR-DGGE techniques (Chapter 3), we found that relative to crop residue management and cropping system factors, tillage has the greatest impact on community composition of soil micro-organisms. This finding is in line with those of other researchers; however, their findings were based on the assessment of specific groups of soil micro-organisms only. For example, Borie et al. (2006) observed higher arbuscular mycorrhizae spore numbers and hyphae length in reduced than in

conventional tillage systems in Chilean ultisols, generally because tillage disrupts fungi mycelia (Kibblewhite et al. 2008). In Brazil, 46 to 216% higher activity of different enzymes has been observed in conservation compared to conventional tillage systems (Balota et al. 2004).

Different techniques must be employed in research in order to reflect well the effects of different management practices on soil organisms. In biological studies, diversity indices such as Simpsons' and Shannon indices are commonly used as composite indicators to characterize biodiversity of various organism groups, and we used this here to characterize the effects of the different management practices on microbial diversity. Although the increase in soil microbial diversity due to reduced tillage as measured by biological indices can be slight (Chapter 3), other activity measures such as N fixation can be significantly enhanced when compared to conventional tillage (Chapter 5). Thus, focusing not only on soil microbial diversity parameters, but also on the functions of specific microbial communities (functional groups) such as nitrogen-fixers is important and can reveal effects not obvious in simpler diversity assessments.

Higher activities of soil organisms in reduced than in conventional tillage are related to the specific composition and abundance of the associated microbial communities. For example, soil fungi associated with scavenging and desorbing P, and supplying that P to legume nodules where it enhances symbiotic N-fixation (Goss and de Varennes 2002), are more abundant at the top soils in reduced than in conventional tillage systems (Simmons and Coleman 2008). More diverse and more efficient N-fixing Bradyrhizobia are found under reduced than under conventional tillage (Ferreira et al. 2000), which may also explain the N fixation results of our study. In general, reduced tillage results in higher bacteria activity (often measured using substrate-induced respiration (Miura et al. 2008)), as well as in higher microbial biomass than in conventionally and intensively tilled systems (Helgason et al. 2009). To my knowledge, the current study is the first to employ advanced non-culture methods to study microbial diversity within reduced tillage systems in East Africa. Certainly more microbial studies are required in conservation tillage systems and with more replications in time, since changes in microbial responses usually occur at different times (Bausenwein et al. 2008), especially with crop rotation cycles.

Reduced tillage does not only improve soil micro-organisms, but also increases soil fauna diversity and abundance. Although the activity of soil macro- and mesofauna was slightly elevated in reduced compared to conventional tillage in our study, greater abundance of soil fauna in reduced than in conventional tillage systems has been well documented in other studies (Kibblewhite et al. 2008; Lenz and Eisenbeis 2000; Marasas et al. 2001). At the same experimental site used in our study, for instance, Ndabamenye (2006) recorded higher termite abundance under reduced than under conventional tillage (44 vs. 18 termites m<sup>-2</sup>). Also, slightly higher residue disappearance in reduced tillage was in line with the slightly higher bacterial diversity and macro- and mesofauna activity when compared to conventional tillage. It is likely, therefore, that the conditions favoring bacteria also favor macro- and mesofauna. Reduced tillage, therefore, has the potential to revive soil biology (fauna and microbes) of degraded soils in Kenya.

The effect of tillage on soil structure has been studied in different parts of the world. On the African Ferralsols, there are few reduced tillage experiments with sufficient replication in time where results can be confidently reported, and even fewer trials are implemented in small-scale farming systems. Reduced tillage results in greater soil macroaggregation (chapter 2), and this is consistent with the slightly improved bacteria diversity and fungal abundance (Chapter 3) compared to conventional tillage. Fungi are primarily responsible for the higher soil macroaggregation in reduced than in conventional tillage systems due to enmeshment of aggregates with their hyphae, and also due to the effects of their extracellular polysaccharides; up to 3 times more active fungal hyphae are found in reduced than in conventional tillage systems (Beare et al. 1997). Relating soil microbial diversity to soil aggregation can reveal how fungi diversity affects the aggregation. At very low diversity, it is likely that fungi species are present that are much more efficient in soil aggregation than fungi species that characterize systems with increasing diversity, only up to a certain threshold where both fungi diversity and soil aggregation increase together (Chapter 3). More studies in this perspective are required to fully elucidate microbial diversity-soil aggregation relationships.

The focus on microaggregates within macroaggregates suggested by Oades (1984) and extensively used by Johan Six and team (Six et al. 2004; Six et al. 1999; Six

et al. 2000a) is a novel approach, which leads to a much better understanding of the aggregation processes and also gives insight into carbon sequestration potential of soils. It is widely reported that microaggregates held within macroaggregates in reduced tillage systems are more stable than in conventional tillage systems (Six et al. 2000a). Our findings, based on five years of reduced tillage, suggest that the microaggregates within macroaggregates are not always more stable than in conventional tillage. This may be related to the time period in which the system has been under reduced tillage management. The less stabilized microaggregates within macroaggregates in reduced tillage foil the greater carbon sequestration expected in this system relative to conventional tillage.

### **7.1.2 Crop residue application**

Soil surface cover with crop residue is a principle of conservation tillage (Derpsch 2008). Management of the residue was identified as one of the areas requiring research in Africa (Fowler and Rockstrom 2001). One of the challenges in Kenya, as in other parts of sub-Saharan Africa, is not only the lack of sufficient quantities of crop residue for application, but also its fast disappearance due to the effect of macro- and mesofauna (Chapter 4). Although in general, biomass production is usually adequate for the needed cover in the sub-humid zone (Chapter 6), the biomass has additional uses, such as for fuel wood, which is contrary to the situation in large-scale production systems and that in the developed world. Nevertheless, despite the modest application (at 2 t ha<sup>-1</sup>) and fast disappearance of the residue, its application under reduced tillage seemed to result in higher soil available P than in non-residue treatments (Figure 7.1; see also Appendix 1), increased maize yields and soybean %NDfA also under reduced tillage and led to greater diversity of soil bacteria. These benefits are related to conserved P, modification of soil microclimate, and provision of nutrients and energy for the soil organisms by the applied crop residue. Phosphorus is protected from losses through runoff in the presence of crop residue, as also reported elsewhere (Andraski et al. 2003).

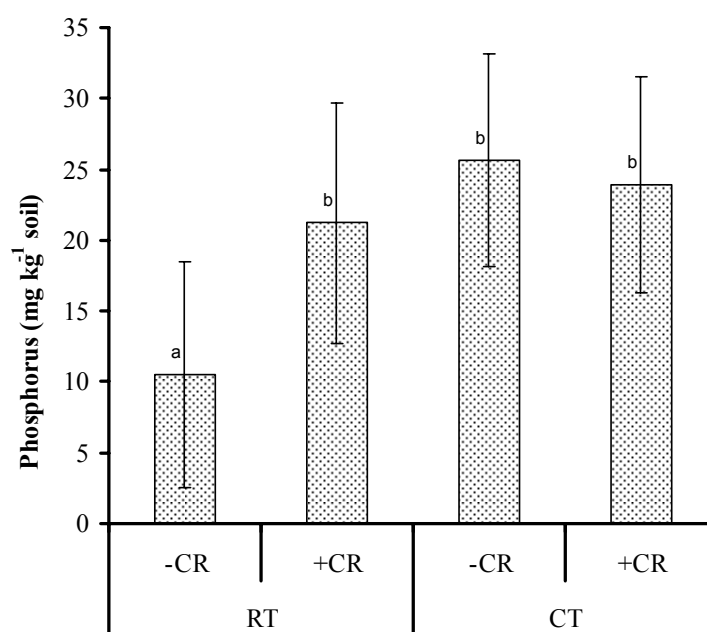


Figure 7.1. Effect of tillage practice on available phosphorus at 0-15 cm soil depth in Nyabeda, western Kenya, March 2007; bars are confidence limits; different letters indicate significant differences; RT=reduced tillage; CT=conventional tillage; CR=crop residue

Crop residue debris are often nuclei for formation of soil aggregates (Plante and McGill 2002). Such soil aggregates are better stabilized in the presence of crop residue, especially at the top 0-5 cm (Chapter 2), because this is the zone of crop residue concentration. Although applied crop residue generally increases fungal growth, i.e., hyphae and biomass (Simmons and Coleman 2008), because it is a carbon source for the fungi, the increases seem to be due to selective growth of few favored fungi species that become dominant when low-quality residues are added, i.e., biological indices (fungi Simpsons' and Shannon indices) are lower than without residue (Chapter 3). The predominating fungi species at the lowered diversity in this study are more efficient in soil aggregation than at slightly higher diversity. Despite the positive benefits of the crop residue (e.g., better soil structure and N fixation), it has been shown that such benefits are optimum at higher rates of residue application. For example, 4 t ha<sup>-1</sup> is determined as the optimum wheat straw mulch rate for increased porosity, while 8 t ha<sup>-1</sup> is optimum for enhanced available water capacity, moisture retention and aggregate stability in a Luvisol in Ohio, USA (Mulumba and Lal 2008). Thus, greater benefits of

improved soil physical, microbial and agronomic performance are very likely to be realized at higher rates of residue application.

The decomposition of low-quality crop residue is usually accompanied by an initial immobilization of available soil N (Jensen 1997), which affects the establishment and growth of maize and slows development of legume nodules (Hansen et al. 1989). The immobilized N released in later crop growth stages suppresses legume dependency on N fixation, and results in lower %NDfA when residue is incorporated than when it is surface-placed. In addition, incorporation of crop residue depresses maize yields in the initial seasons (up to season 6 in our case (Chapter 6)) but decreases significantly %NDfA even in later seasons, e.g., during the season 9 (Chapter 5). Thus, even though the deleterious effect of crop residue incorporation on harvest maize yield can disappear after the first 6 seasons, the effect on specific functioning of soil organisms can extend for much longer. As also bacteria diversity was positively influenced by incorporated crop residue (Chapter 3), more research is needed focusing on the effects of incorporated crop residue on other microbial functions before firm conclusions can be made. Nevertheless, surface placement of crop residue is recommended in this study; the residue applied should combine low- and high-quality components in order to improve also diversity of fungi.

Crop yield is the most important component for the farmer. The combination of the two main requirements of conservation tillage, i.e., reducing tillage and applying crop residue, is normally expected to result in similar or higher yields relative to conventional tillage systems. Unfortunately, there are cases where the yields in ‘reduced tillage plus crop residue’ are not improved and are lower than in conventional tillage systems especially during the initial seasons as was observed in this study (Chapter 6 and Appendix 2). The slightly lower maize yield in ‘reduced tillage plus crop residue’ (for 7-9 seasons) than in conventional tillage contrasted with the higher aggregation, microbial diversity and N fixation observed in season 9. Similar to the findings of studies integrating multiple long-term trials (Wang et al. 2006; West and Post 2002), benefits of reduced tillage are translated into maize yields similar to those in the conventional tillage system after some seasons of initially lower yields. Surface crusting of the Ferralsols of western Kenya is identified as the major drawback to the translation of improved soil structure and soil biology into similar or higher maize yields in

reduced compared to conventional tillage, and addressing it is suggested by this study as a priority issue.

The low maize yields in the reduced compared to the conventional tillage system, which can be an impediment to the adoption of reduced tillage (Taa et al. 2004), can be related to the lack of sufficient and permanent soil cover in the ‘reduced tillage plus crop residue’ fields. It seems challenging in small-holder farming systems that such permanent soil cover will derive solely from crop residues after harvest. Additional options for soil cover between cropping seasons such as green manure cover crops need to be investigated. Such green manure cover is not likely to suffer as severe termite attacks as the maize stover, because the green manure provides live cover. Strategies to boost soil cover can also result in even greater savings in labor, which results from the lower investments in weed management required under reduced tillage, and in the greater net benefits than found under conventional tillage.

One finding regarding soybean yields is interesting: soybean yields were always similar between reduced and conventional tillage, suggesting no need to use conventional tillage in soybean production. Similar findings with soybean yields have been observed in Canada (Légère et al. 2008), in USA (Lyon et al. 2004) and in Brazil (Silveira and Stone 2003), but lower soybean yields in reduced tillage systems than conventional tillage have also been reported, e.g., in Italy (Mazzoncini et al. 2008). Considering the similar soybean yields and the environmental benefits resulting from improved soil structure and enhanced microbial communities (Chapter 2, 3 and 5, see also Blank 2008), reduced tillage offers a better alternative to conventional tillage. However, the challenge of the initially lower maize yields need to be addressed in future studies.

### **7.1.3 Cropping systems**

The cropping systems commonly used by farmers have significant influences on soil physical, chemical and biological parameters as well as on overall system economic performance. In addition to soil organic matter, plant roots in the different cropping systems and their associated fungal hyphae enmesh and stabilize soil macroaggregates (Tisdall and Oades 1982). Crop mixtures (soybean/ maize intercropping) result in greater diversity of organic resources such as varied root residues and organic exudates,



have greater microbial richness (Chapter 3), and result in higher soil macroaggregation (Chapter 2) than maize and soybean monocropping systems. Soil aggregation and microbial diversity complement each other. For example, higher macroaggregation leads to better preservation of soil bacteria due to reduced predation by protozoa (Six et al. 2004). On the other hand, the diverse microbial communities contribute to stabilization of microaggregates within macroaggregates through the effects of their extracellular polysaccharides (Beare et al. 1997; Degens 1997). This is perhaps why the system of highest soil macroaggregation in Nyabeda (soybean/maize intercropping) also showed highest microbial diversity (see Chapter 2 and 3). The contribution of microbes to soil macroaggregation was confirmed by the higher hot water-extractable carbon in intercropping than in the other cropping systems (Chapter 2). Such hot water-extractable carbon has been shown to correlate well with soil aggregate stability (Yousefi et al. 2008) and is usually of microbial origin (Degens 1997). Interestingly also, the highest economic benefits were in the intercropping system as the plants in this system optimized available resources and benefited from the improved biological and physical environments. This is in agreement with the higher land equivalence ratios often reported for intercropping relative to rotation and continuous cereal systems (Diangar et al. 2004; Worku 2004). Unfortunately, the intercropping system has received only little attention in research (perhaps because it is complicated and also labor-consuming for the farmer), and only recently has some researchers started to incorporate it into the definition of the 'rotation principle' of conservation agriculture.

Separating the crop components in time (rotation) does not benefit macroaggregate formation over the continuous maize monocropping system, as macroaggregates formed during the soybean phase likely break up during the cereal phase. In general, easily decomposable organic resources such as soybean residue have an intense and transient effect on aggregate stability, while more recalcitrant organic resources such as maize residue have a lower but longer term effect (Abiven et al. 2009). The cropping systems represent different residue quality, and substrate decomposability is a key factor determining extent and dynamics of macroaggregation during decomposition processes; amending soil with maize leaves resulted in more rapid maximum macroaggregation than amendment with maize roots (Helfrich et al. 2008). Because of its fast decomposition (up to 70% biomass can decompose in 5 weeks

(Thönnissen et al. 2000)), soybean biomass likely strongly influences r-selected microorganisms, which die off as also macroaggregates formed on soybean residue disintegrate. Because crop rotation is also the most economically attractive tillage system as indicated by the dominance analysis and marginal rate of return (Chapter 6), it is likely that the temporal separation of crop components will continue, and its long-term impact on soil aggregation in the Ferralsols of western Kenya should be investigated.

Studies on termite activity have typically focused on forest ecosystems and, when outside forests, off-farm mounds have been the central sampling points. Our study is among the very few studies that have considered termite galleries (mainly sheetings) within farm fields. The sheetings are more enriched in carbon than the surrounding bulk farm soils. The volume of soils in termite sheetings was earlier estimated to be up to 1.3 t ha<sup>-1</sup> year<sup>-1</sup> (Kooyman and Onck 1987b), but the agronomic impact can be much greater, as microbial diversity in termite soils is also likely affected. Termites scavenge sheeting soils from surrounding soils, and as such the sheeting soils bear some similarity with the surrounding soils, unless the aggregate in the surrounding soils are larger than the carrying capacity of termites (Kooyman and Onck 1987b). Surface termite activity is higher under monocrop maize than under soybean (Chapter 4), but we suggest that future assessment be spread over the seasons to capture the different crop stages.

This study targeted small-scale farmers. In this group, growth of conservation agriculture has been estimated to be much lower than that of mechanized systems, since very little research and development as well as extension efforts are accorded to these farmers worldwide (Derpsch 2008). Our findings show a strong case that reduced tillage promotes ecosystem health when compared to conventional tillage system. The urgent need is to eliminate soil surface crusting in reduced tillage by providing greater soil surface cover than the 2 t ha<sup>-1</sup> maize stover used in order to effectively translate the improved soil structure and greater soil biology into higher maize yields than in conventional tillage. The cost of labor should also be reduced in order to make reduced tillage economically more attractive for the farmers.

## 7.2 Summary

Practicing reduced tillage leads to higher fractions of macroaggregates but to lower fractions of microaggregates protected within macroaggregates compared with conventionally tilled systems under periodic disturbance. Also, intercropping soybean and maize leads to better soil aggregation than continuous cereal monocropping. The highest soil macroaggregation is achieved when reduced tillage plus crop residue is practiced in intercropping systems.

Tillage affected soil microbes and led to different bacteria and fungal community composition in reduced and conventional tillage. Crop residue application influenced bacteria species composition more under reduced than under conventional tillage. However, crop residue slightly reduced diversity of fungi. Microbial diversity was greatest in intercropping followed by rotation and was lowest in continuous maize. For fungi Simpsons' indices  $<0.7$ , fungi species present at low diversity indices were associated with greater soil aggregation than the species at improved diversity.

Macro- and mesofauna contribute to greater residue loss in western Kenya than other biotic and abiotic factors. In the presence of macro- and mesofauna, the rate of disappearance of applied residue (maize stover) is similar for surface-placed and buried residue. Termite-molded surface gallery/sheeting soil has higher carbon content and similar aggregate size distribution when compared to the bulk farm soil. Nesting material of the termites has highly elevated macroaggregate sized soils than the bulk farm soil, but their carbon content is lower because the soil is imported from low carbon subsoil.

Soybean N derived from biological fixation (%NDfA) and N fixed are higher in 'reduced tillage plus crop residue' than in 'reduced tillage minus crop residue' and than in conventional tillage systems, because of greater fungal growth and hence better P nutrition in the 'reduced tillage plus crop residue' system.

Soybean yield is similar in both reduced and conventional tillage. Lower maize yield in reduced tillage before and similar yield after season 6 was observed compared to conventional tillage. Nitrogen and P fertilizer application is important because of the low nutrient status of the soils. This results in higher (by up to 3 times) maize and soybean yields relative to no-input control, which is often the farmers practice.

### 7.3 Recommendations

Long-term experiments, as used in this study, are necessary to understand the processes influencing the effectiveness of reduced tillage. Although the maize yields are lower in reduced tillage compared to conventional tillage, this study demonstrates that ‘reduced tillage plus crop residue’ offers the opportunity to revive the biology of the degraded farmlands. The following recommendations are made:

- ‘Reduced tillage plus crop residue’ ultimately leads to greater benefits from biological nitrogen fixation for the farmer, and greater microbial diversity and better soil structure, and has similar soybean yields to those of the conventional tillage system. Soybean production in western Kenya is, therefore, better undertaken under reduced tillage.
- Practicing reduced tillage with and without 2 t ha<sup>-1</sup> crop residue results in lower maize yields than conventional tillage during the first six seasons; the maize yields are similar thereafter, and farmers should be made aware of this when reduced tillage is being promoted.
- Reduced tillage should always be accompanied by application of crop residue as surface mulch to avoid substantial losses in soil nutrients such as P, and reduced maize crop yields.
- Since the amount of crop residue for use as surface cover in reduced tillage systems is inadequate, opportunities to increase soil cover need to be explored.

The following research questions are suggested for future investigations:

- 1) the influence of crop residue and manure on specific fungi species or phylogenetic groups,
- 2) relation of microbial diversity and soil aggregation so that cross site comparisons can be made,
- 3) comparison not only of yield but also of physical, chemical and biological conditions between intercropping and rotation systems in multiple sites.

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## 9 APPENDIX

Appendix 1. Soil chemical characteristics (0-15 cm) in different tillage, crop residue and cropping systems in Nyabeda, western Kenya, 2007-2008

<i>Tillage/residue management</i>	<i>Cropping system</i>	<i>pH (water)</i>	<i>% SOC</i>	<i>%N*</i>	<i>K (me 100g soil<sup>-1</sup>)</i>	<i>P (mg kg soil<sup>-1</sup>)</i>
CT-CR	Continuous	5.02 <sup>a</sup>	1.37 <sup>a</sup>	0.147 <sup>a</sup>	0.16 <sup>a</sup>	14.17 <sup>a</sup>
CT-CR	Intercrop	5.08 <sup>a</sup>	1.45 <sup>a</sup>	0.149 <sup>a</sup>	0.21 <sup>a</sup>	29.11 <sup>a</sup>
CT-CR	Rotation	5.08 <sup>a</sup>	1.38 <sup>a</sup>	0.149 <sup>a</sup>	0.19 <sup>a</sup>	32.61 <sup>a</sup>
CT+CR	Continuous	5.14 <sup>a</sup>	1.33 <sup>a</sup>	0.147 <sup>a</sup>	0.17 <sup>a</sup>	22.92 <sup>a</sup>
CT+CR	Intercrop	5.07 <sup>a</sup>	1.34 <sup>a</sup>	0.149 <sup>a</sup>	0.20 <sup>a</sup>	25.59 <sup>a</sup>
CT+CR	Rotation	5.04 <sup>a</sup>	1.37 <sup>a</sup>	0.149 <sup>a</sup>	0.22 <sup>a</sup>	21.93 <sup>a</sup>
<i>Average</i>		<i>5.07</i>	<i>1.37</i>	<i>0.148</i>	<i>0.19</i>	<i>24.39</i>
<i>SE</i>		<i>0.072</i>	<i>0.053</i>	<i>0.0044</i>	<i>0.0366</i>	<i>6.916</i>
RT-CR	Continuous	5.03 <sup>a</sup>	1.29 <sup>a</sup>	0.143 <sup>a</sup>	0.23 <sup>b</sup>	4.29 <sup>a</sup>
RT-CR	Intercrop	4.93 <sup>a</sup>	1.36 <sup>a</sup>	0.150 <sup>ab</sup>	0.31 <sup>b</sup>	14.03 <sup>ab</sup>
RT-CR	Rotation	5.19 <sup>b</sup>	1.36 <sup>a</sup>	0.145 <sup>a</sup>	0.10 <sup>a</sup>	12.19 <sup>ab</sup>
RT+CR	Continuous	5.10 <sup>ab</sup>	1.37 <sup>a</sup>	0.147 <sup>ab</sup>	0.21 <sup>ab</sup>	22.50 <sup>b</sup>
RT+CR	Intercrop	5.11 <sup>ab</sup>	1.36 <sup>a</sup>	0.153 <sup>b</sup>	0.21 <sup>ab</sup>	14.22 <sup>ab</sup>
RT+CR	Rotation	5.13 <sup>ab</sup>	1.36 <sup>a</sup>	0.147 <sup>ab</sup>	0.30 <sup>b</sup>	24.74 <sup>b</sup>
<i>Average</i>		<i>5.08</i>	<i>1.35</i>	<i>0.147</i>	<i>0.23</i>	<i>15.33</i>
<i>SE</i>		<i>0.078</i>	<i>0.059</i>	<i>0.0033</i>	<i>0.044</i>	<i>6.016</i>
<i>SE across tillage system</i>		<i>0.072</i>	<i>0.056</i>	<i>0.0039</i>	<i>0.041</i>	<i>6.123</i>

CT= conventional tillage; RT= reduced tillage; CR= crop residue; SE= standard error of means; values in the same column and tillage system followed by different letters are significantly different; pH, SOC, K and P were sampled after 10 seasons (February 2008) of experimentation, while sampling for N was in March 2007

## Appendix

Appendix 2. Maize grain and stover yield for first (2-5) and last (7-9) seasons as observed in Nyabeda, western Kenya between March 2003 and August 2007

<i>Treatment</i>	<i>CM</i>	<i>Rot</i>	<i>Int</i>	<i>CM</i>	<i>Rot</i>	<i>Int</i>
	Grain yield (t ha <sup>-1</sup> )			Stover yield (t ha <sup>-1</sup> )		
---Seasons 2-5---						
Reduced tillage -CR	3.47 <sup> b</sup>	3.44 <sup> b</sup>	1.61 <sup> b</sup>	3.82 <sup> bc</sup>	3.97 <sup> bc</sup>	2.38 <sup> b</sup>
Reduced tillage +CR	3.89 <sup> b</sup>	3.27 <sup> b</sup>	1.82 <sup> b</sup>	4.60 <sup> ad</sup>	3.55 <sup> b</sup>	2.57 <sup> b</sup>
Conventional tillage -CR	4.55 <sup> a</sup>	4.68 <sup> a</sup>	2.91 <sup> a</sup>	5.07 <sup> a</sup>	4.86 <sup> a</sup>	3.18 <sup> a</sup>
Conventional tillage +CR	4.52 <sup> a</sup>	4.30 <sup> a</sup>	2.67 <sup> a</sup>	4.52 <sup> acd</sup>	4.45 <sup> ac</sup>	3.11 <sup> a</sup>
<i>SE</i>	<i>0.503</i>	<i>0.559</i>	<i>0.394</i>	<i>0.547</i>	<i>0.424</i>	<i>0.45</i>
<i>Tillage</i>	<b>**</b>	<b>***</b>	<b>***</b>	<b>*</b>	<b>**</b>	<b>**</b>
<i>Tillage x crop residue</i>				<b>**</b>		
---Seasons 7-9---						
Reduced tillage -CR	3.16 <sup> a</sup>	3.01 <sup> a</sup>	1.86 <sup> b</sup>	3.59 <sup> ab</sup>	3.08 <sup> b</sup>	2.53 <sup> b</sup>
Reduced tillage +CR	3.29 <sup> a</sup>	2.95 <sup> a</sup>	2.10 <sup> bc</sup>	3.25 <sup> b</sup>	3.13 <sup> ab</sup>	2.28 <sup> b</sup>
Conventional tillage -CR	3.49 <sup> a</sup>	3.29 <sup> a</sup>	2.61 <sup> a</sup>	4.08 <sup> a</sup>	3.55 <sup> ab</sup>	3.44 <sup> a</sup>
Conventional tillage +CR	3.75 <sup> a</sup>	3.47 <sup> a</sup>	2.52 <sup> ac</sup>	3.98 <sup> ab</sup>	3.76 <sup> a</sup>	2.79 <sup> b</sup>
<i>SE</i>	<i>0.467</i>	<i>0.595</i>	<i>0.421</i>	<i>0.574</i>	<i>0.666</i>	<i>0.621</i>
<i>Tillage</i>			<b>*</b>	<b>*</b>	<b>*</b>	<b>*</b>

CM=continuous maize, Rot=soybean-maize rotation, Int= soybean/ maize intercropping, CR=crop residue; values in the same column and assessment period followed by the same letter are not significantly different at P<0.05; SE= standard error; \*significant at P<0.05, \*\*significant at P<0.01, \*\*\*significant at P<0.001

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