IMPACTS OF CROPPING SYSTEMS ON SOIL HEALTH
AND MICROBIAL ECOLOGY

by

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Abstract

Declining soil health is the underlying cause of decreasing agricultural productivity and environmental degradation. To address this challenge, research was conducted to determine how: (1) cover crops affect soil health in Kansas, USA and (2) direct seeding mulch–based cropping (DMC) systems affect soil health in Nyankpala, Ghana. Soil health indicators assessed include: biomass yield (kg ha^{-1}), soil microbial respiration (SMR), soil microbial C and N (MBC & MBN), potentially mineralizable N (PMN), dissolved organic carbon (DOC), soil organic C (SOC), soil total nitrogen (TN), phospholipid fatty acid analysis (PLFA), water stable aggregate (WSA), bulk density, pH, N, P, K, Ca and Mg. DMC systems from Ghana yielded significantly greater biomass compared to the control. High biomass produced by DMC systems did not increase SOC and PMN relative to the control. Fertilizer application had a significant impact on biomass production, which resulted in a significant increase in SOC and PMN in the 0-5 cm soil layer. Soil pH was significantly reduced by cropping systems and fertilizer in the 0-5 cm soil layer. Microbial biomass N, TN, SMR, N, P, Ca and Mg were not affected by the DMC cropping systems. Application of mineral fertilizer increased SMR, MBN, TN, N, and P. Soil K was also significantly affected by cropping systems and mineral fertilizer. The combination of mineral fertilizer and plant residues would be needed to improve soil health and increase crop productivity in the Guinea Savanna Zone of Ghana. Liming would be required to address low soil pH. In the USA, of all the soil health indicators examined, actinomycetes, gram-positive bacteria, fungi-bacteria ratio (F:B), SMR, MBN and WSA, were those significantly influenced by cover crops. The interactive effect of cover cops and N fertilizer also affected gram-positive bacteria, total PLFA, MBN, F:B ratio and WSA. Cover crop residues contributed to the observed differences in these indicators. The low response of soil health indicators suggest further evaluations are needed to determine the effectiveness of the indicators.

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Dedication

I dedicate this work to:

My wife (Gladys Dogbah) and my children (Lillan A. Woyram Akley and Gabriel K. Aseye Akley) for their love and sacrifice.

My parents for laying the foundation of my education.

Chapter 1 - Introduction & Literature View

Soil health holds the key to sustainable food production to feed the increasing human population. Soil health is defined as continued capacity of the soil to function as a living system within natural ecosystem boundaries, to sustain biological and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Carter et al., 1997; Arshad and Martin, 2002; Shukla et al., 2006; Karlen et al., 2008). Weil and Magdoff (2004) stated the functions of a healthy soil include: (1) producing healthy plants; (2) cycling and retention of nutrients such as N ,P, K and C sequestration; (3) providing habitat for soil organisms and serving as a reservoir for biodiversity; (4) supplying plants and soil organisms with air and water for survival; (5) maintaining water quality and protecting it from contamination by nutrient and pathogens; (6) providing physical support for vegetation; (7) buffering against toxic accumulation and transport of natural and synthetic compounds; and (8) finally filtering elements to protect animals, plants, and the environment from undesirable exposure. These attributes are influenced by agricultural management practices.

Assessment of soil health has been a challenge because there are no minimum data sets. Presently, assessments are made from management-induced changes in soil attributes (Bhardwaj et al., 2011). Over the years, research on soil health assessment focused mainly on soil chemical and physical characteristics because simple methods of analysis were available (Larson and Pierce, 1991; Carter et al., 1997; Bhardwaj et al., 2011). During those periods, soil biology did not receive much attention because soil biological measurements were difficult to make and interpret due to their sensitivity to dynamic changes related to daily changes in the soil environment (Pankhurst et al., 1997; Bhardwaj et al., 2011). However, several recent studies have recognized the need to include soil biology as an important indicator of soil health

(Kennedy and Papendick, 1995; Bhardwaj et al., 2011; Biswas et al., 2014). This is because soil biology plays important roles in nutrient cycling, soil aggregation and soil structure development. At present the available soil heath indicators that are used include organic carbon, microbial biomass, potentially mineralizable nitrogen, soil respiration and enzyme activity (Larson and Pierce, 1994; Kennedy and Papendick, 1995). Others include ratio of biomass carbon to total organic carbon, ratio of microbial respiration to microbial biomass, microbial substrate utilization, fatty acid analysis, nucleic acid analysis, and substrate utilization (Larson and Pierce, 1994; Kennedy and Papendick, 1995). Moreover, several authors have suggested that any proposed soil health indicator(s) should be directly linked to soil function (Larson and Pierce, 1994; Acton and Gregorich, 1995; Doran et al., 1994; Karlen et al., 1997; Pankhurst et al., 1997; Bhardwaj et al., 2011). To date, no appropriate standard of data interpretation has been developed to meet the suggested general guidelines (Bhardwaj et al., 2011).

Soil Respiration

Soil respiration is one of the most frequently used biological indicators for soil health. It indicates microbial activity and mineralization of labile C in the soil (Parkin et al., 1996; Allen et al., 2011). Microbial activity is a fundamental process that makes energy and nutrients available for recycling in an ecosystem. This is because soil microorganisms play crucial roles in the bio-geochemical cycling of organic C, N, P, K, S, etc. (Bandick and Dick, 1999; Schoenholtz et al., 2000). High microbial respiration indicates loss of organic C and low nutrient cycling in the soil (Alef, 1995; Pankhurst et al., 1997). Whereas low microbial respiration indicates immobilization and /or the presence of pollutants such as fungicides or pesticides (Pankhurst et al., 1997). Soil microbial respiration has a linear relationship with soil organic matter (SOM) mineralization. Respiration is estimated as either CO_2 production or O_2 consumption, using basal respiration

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such as short-term laboratory assays (Anderson and Domsch, 1993; Parkin et al., 1996; Anderson and Jensen, 2001). In general, soil microbial respiration is affected by changes in precipitation, management practice, microbial community structure, aeration, soil structure, nutrient conditions and pH (Anderson and Domsch., 1993; Schloter et al., 2003; Singh et al., 2011). In addition, respiration is temperature sensitive and has a close relationship with climate change and global C cycling (Chou et al., 2008; Wixon and Balser, 2009; Singh et al., 2011).

Soil microbial biomass

Microbial biomass carbon (MBC) is a small fraction of the soil labile C that is biologically significant and sensitive to management practices (Bolton et al., 1985; Powlson, 1994; Schloter et al., 2003; Haynes, 2008; Singh et al., 2011). As an indicator of soil health and soil fertility, MBC serves as a reservoir of nutrients (N, P and S) (Dick 1992; Schutter and Dick, 2002; Haubensak et al., 2002). Research has shown that MBC has a linear correlation with SOM (Gregorich et al., 1994; Carter et al., 1997; Gregorich et al., 1997). Microbial biomass N (MBN) indicates potentially available N which is a significant source or sink for N. Nitrogen present in the microbial biomass is part of a larger pool of potentially mineralizable N that is available to plants (Sainju et al., 2008). Hence MBN has also been connected with N mineralization to estimate the quality of organic matter (Allen et al., 2011). The quantity of microbial biomass in the soil is influenced by crop residues, root biomass, nutrient amendment, C, N, soil pH, concentration of heavy metals and pesticides, clay content, soil water content, and temperature (Haubensak et al., 2002; Allen et al., 2011). Seasonal fluctuations due to changes in climate conditions affect microbial biomass. For instance, Dalal (1998) observed that an increase in annual precipitation increased microbial biomass, whereas an increase in annual temperature decreased microbial biomass. Rinnan et al. (2007) also found a significant decline in microbial

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biomass during long term simulated climate warming. Soil microbial biomass, similar to labile C, is responsive to short–term environmental change (Haynes and Beare, 1997; Haynes, 2008)

There are many methods used to measure microbial biomass in soil. This includes direct microscopic counting, chloroform fumigation incubation (CFI) (Jenkinson and Powlson, 1976; Jenkinson and Powlson, 1980), chloroform fumigation extraction (CFE) (Brookes et al., 1987; Vance et al., 1987; Vance and Chapin III, 2001), substrate induced respiration (SIR) (Anderson and Domsch, 1978; Anderson and Ineson, 1982; Anderson and Domsch, 1993), extraction of ATP (Brookes et al., 1987), total extractable phospholipid fatty acids (PLFA) or extraction of DNA (Schloter et al., 2003; Steenwerth et al., 2003). However, CFI is the most commonly used technique. Singh et al. (2011) reported that microbial C measures combined with ^{13}C isotope labelling techniques provides information on shifts in microbial community structure. The challenges associated with MBC as an indicator of soil health is that there is no appropriate universal benchmark which hinders interpretation (Dalal, 1998).

Potentially Mineralizable Nitrogen

Potentially mineralizable N (PMN) is the quantity of organically bound N mineralized by the soil microbial community into inorganic N (Gugino et al., 2009; Wang, et al., 2009). Biological activity of the soil is regarded as an indicator of PMN (Hirzel et al., 2012). PMN is considered to be either a single pool of N, or divided into three components, biomass N, active non-biomass N, or stabilized N (Duxbury and Nkambule, 1994; Hirzel et al., 2012). PMN is an important indicator of soil health when used in combination with total N, total C, or microbial biomass (Gugino et al., 2009). Most soils contain large amounts of organic N, however, a significant fraction of this N is chemically or physically stabilized and resistant to microbial degradation (Stanford and Smith, 1972; Moron and Cozzolino, 2004). Only a small fraction of

this organic N is labile and serves as substrate for N mineralization (Stanford and Smith, 1972). The release of N from organic sources is dependent on mineralization rates (Moron and Cozzolino, 2004). The labile N pool varies in size depending on the soil type. Mineralizable N is influenced by crop management and amendment history, biotic and abiotic soil characteristics, and environmental factors including soil temperature and water content (Griffiths et al., 2010; Allen et al., 2011). In addition, factors controlling N mineralization depends on organic C, soil pH, CEC, clay, silt, and extractable P, Mn, and Zn (Narteh and Sahrawat, 1997). The quantity of PMN is dependent on the quality of SOM acting as an interface between autotrophic and heterotrophic soil organisms during nutrient cycling (Gregorich et al., 1994; Allen et al., 2011)

Phospholipid Fatty Acid Analysis

Phospholipids ester-linked fatty acids (PLFA) serve as an indicator of soil health and/or environmental stress to provide information on diversity of the microbial community. It can be a powerful tool to measure changes in microbial community structure resulting from management practices (Frostegård et al., 1993; Bååth et al., 1995). PLFA analysis uses the membrane lipids within microorganisms, as biomarkers for specific groups of organisms which create a profile of the microbial community (Frostegård et al., 1991; Steenwerth et al., 2003; Frostegård et al., 2011). Fatty acids are unique biomarkers for specific microbial community groups (Frostegård et al., 1993; Bååth and Anderson, 2003). The PLFA technique is more sensitive and culture independent compared to traditional methods like plate counts techniques. Total PLFA is an indicator of viable microbial biomass (Zelles, 1997; Steenwerth et al., 2003; Frostegård et al., 2011; Schmitt and Glaser, 2011). Microbial community structure is distinguished by the presence and availability of a particular biomarkers (Zelles et al., 1992; Frostegård et al., 1993; Cavigelli et al., 1995; Zelles, 1997; Zelles, 1999; Schmitt and Glaser, 2011). Gram-negative bacteria

normally have monounsaturated fatty acids biomarker: 16:1ω7c, 18:1ω7c (Zelles et al., 1992; Zelles, 1999; Frostegård et al., 2011). Actinomycetes have mid-chain branched saturated fatty acids biomarkers: 10Me16:0, 10Me17:0, 10Me18:0 (Steenwerth et al., 2003; Shi et al., 2006). Gram-positive bacteria typically have terminal-branched saturated fatty acids biomarkers: a15:0, i15:0, i16:0, i17:0, a17:0 (Zelles, 1999; Schmitt and Glaser, 2011). While short -chain saturated biomarkers: 14:0, 15:0, 16:0, 17:0,18:0, and cyclopropyl saturated biomarker: cy17:0, cy19:0 are generally considered presence in all microorganisms, hence they are regarded as non-specific bacterial biomarkers (Bååth and Anderson, 2003; Steenwerth et al., 2003; Schmitt and Glaser, 2011). Typical biomarkers for fungi are: 18:2ω6, 9, 18:1ω9c and 16:1ω5c (Zelles, 1999; Myers et al., 2001; Waldrop et al., 2004; McMahon et al., 2005). The biomarker, 16:1ω5c, is associated with arbuscular mycorrhiza fungi. The fungal:bacterial ratio in soils has been associated with C sequestration potential (Trivedi et al., 2013). Higher fungal abundance indicates greater C sequestration potential (Trivedi et al, 2013; Ghimire et al., 2014) and aggregation. Soil pH, soil C:N, and soil management practices affect the fungal:bacterial ratio in the soil (Ghimire et al., 2014).

Soil Organic Carbon

Soil organic carbon (SOC) influences soil fertility, stability of aggregates, and erosion. SOC, as indicator of soil health is directly linked to changes that occur within an ecosystem over time (Schoenholtz et al., 2000; Lal, 2004). SOC plays a significant role in the carbon cycle and as a source of nutrients as it affects soil biological and chemical processes (Schloter et al., 2003; Lal, 2004). Human activities influence SOC levels. The level of SOC is influenced by many factors such as water availability, pH, temperature, oxygen supply, and drainage (Schoenholtz et al., 2000; Allen et al., 2011). Others include are nutrient supply, clay content and mineralogy

(Brejda et al., 2000a; Six et al., 2004). Losses of SOC is controlled by increased tillage, microbial mineralization or decomposition, soil erosion, bush fires, and residual removal (Schoenholtz et al., 2000; La1, 2004). Changes in SOC are largely responsible for the variation in the chemical, physical and biological properties of the soil (Schoenholtz et al., 2000; Mazzoncini et al., 2011). Therefore, Larson and Pierce (1991) stated organic C should be included in the minimum data set of soil health assessment for agricultural soils.

Soil pH

Soil pH is a function of parent material, time of weathering, vegetation, and climate (Allen et al., 2011). Soil pH measures the degree of acidity and alkalinity of soil and as an indicator of soil health, it affects nutrient availability, toxicity and microbial activity (Schoenholtz et al., 2000; Bronick and Lal, 2005; Lehman et al., 2015). Nitrification of ammonium leads to a decline in soil pH. Sources of ammonium include the application of ammonium-based fertilizers, and mineralization of organic N whether from SOM, biological N fixation, or plant residues (Rengel, 2004; Rengel, 2011; Peoples et al., 2004). Soil pH is one factor that influences the microbial community as different microorganisms exhibit different tolerances to pH (Lehman et al., 2015). Liming raises the soil pH to an acceptable level for crop production (Bronick and Lal, 2005). Liming increases microbial activity and decomposition of SOM (Bronick and Lal, 2005) Liming often improves soil structure and aggregation, and increases crop yields. Furthermore, crop management and fertilizer application can affects soil pH either positively or negatively (Schoenholtz et al., 2000).

Soil Phosphorus

Soil phosphorus (P) plays important roles in plant growth and development. Available P affects root and shoot growth, and seed formation. It also increases crop yields and biomass

production (Schoenholtz et al., 2000; Bronick and Lal, 2005). Soil available P influences colonization of arbuscular mycorrhiza fungi, which affects soil aggregation and root morphology (Bronick and Lal, 2005). Soil amendments such as the application of chemical fertilizer, animal manure and surface organic residue make P available for crop growth.

Soil Bulk Density

Soil bulk density is defined as the ratio of oven-dried soil weight to its bulk volume. In general, most agricultural soils have bulk densities ranging from 1.3 to 1.7g cm^{-3}. Soil bulk density varies with soil type, texture, structure and organic matter (Schoenholtz et al., 2000). Cropping systems that provide more organic matter have a significant impact on soil bulk density (Schoenholtz et al., 2000; Logsdon and Karlen, 2004). Bulk density has a negative relationship with SOM (Logsdon and Karlen, 2004; Weil and Madoff, 2004). This infers that as SOM decreases, bulk density increases. Bulk density can be an indicator of soil compaction (Logsdon and Karlen, 2004). Soil bulk density, as an indicator of soil health, affects soil aeration, water infiltration, and water retention properties (Reynolds et al., 2002; Pattison et al., 2008; Allen et al., 2011). Bulk density also affects rooting depth, plant nutrient availability, and soil microbial activity (USDA-NRCS, 2003).

Aggregate Stability

Soil aggregates consist of soil particles bound together resulting from the interaction of soil biota and plant community, and their products with soil mineral components (Allen et al., 2011). Aggregate stability is the resistance of soil aggregates to maintain integrity when exposed to external energy such as high intensity rainfall or cultivation. Aggregate stability is influenced by soil chemical and biological properties. In addition soil structure and management practices can significantly affect aggregate stability (Dalal and Moloney, 2000; Moebius et al., 2007). Wet

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aggregate stability (WAS) indicates how well soil particles can resist the impact of rain drops and water erosion. Whereas size distribution of dry aggregates is used to predict soil resistance to abrasion and wind erosion. Aggregate stability contributes to several aspects of soil health. Aggregate stability improves soil aeration and water drainage and retention. Aggregate stability offers physical protection for soil organic C. (Six et al., 2004; Bronick and Lal, 2005). Aggregate stability prevents soil erosion and enhances plant root development (Six et al., 2004; Bronick and Lal, 2005; Tate et al., 2007). Changes in aggregate stability may serve as an early indicator of soil recovery or degradation.

Aggregate sizes are closely related to the amount of nutrients and their turnover. Macroaggregates (>250 μm) are formed and bound by biological agents, such as roots, fungal hyphae and microbial by-products (Tisdall and Oades, 1982; Karlen et al., 1992; Oades, 1993; Karlen et al., 1994; Lado et al., 2004; Smith et al., 2014). While microaggregates are usually formed and bound by chemical agents, such as clay mineralogy (Tisdall and Oades, 1982; Oades, 1993; Six et al., 2004). Macroaggregates contain higher concentrations of C and N relative to microaggregates (Mikha and Rice, 2004), however, some authors found the contrary (He et al., 1995; Maguire et al., 1998). Six et al. (2000) observed that the turnover rates of SOC are higher in macroaggregates compared to microaggregates. Soil management, especially tillage systems, directly affects soil aggregation by causing physical disruption to macroaggregates. Indirectly, aggregates alter the biological and chemical properties of the soil (Barto et al., 2010). Decreases in aggregate stability result in depletion of nutrients and soil erosion (La1, 2004; Borie et al., 2006). Cover crops, green manure crops, residue management, and reduced soil disturbance can improve aggregate stability (Six et al., 2004; Lal, 2004).

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Effect of Management

Sustainable agricultural management practices such as no-till and cover crops affect soil health. Cover crops are an important component of sustainable agricultural systems because they help in soil conservation. Cover crops are crops grown to serve as green manure, living mulches, residue mulches, catch crops, and forages. They are usually terminated before the main crop is grown. The decision on which cover crop species to plant depends on the farmers expected outcomes such as controlling soil erosion and pests, increasing nutrients, adding C, and increasing profits (Hartwig et al., 2002). In addition, soil type, climate, and the crop preceding the cover crop are also taken into account (Hartwig et al., 2002; Sarrantonio and Gallandt, 2003). The specific benefits associated with cover crops vary by species and management practices. Brassicas, grasses, and legumes are the three major groups of cover crops commonly grown. Grasses normally produce biomass that are resistant to decomposition. Sarrantonio and Gallandt (2003) reported that in the Northern USA, cereal rye (*Secale cereale*) was one of the most useful grass species for controlling soil erosion due to its deep and fibrous root system. In addition, cereal rye has the capacity to quickly germinate and is tolerant to cool weather (Sarrantonio and Gallandt, 2003). In general, grasses have the capacity to increase soil organic C. Species in the legume family (Fabaceae or Leguminosae) are typically used as cover crops because of their ability to utilize atmospheric N through a mutualistic association with N-fixing bacteria (Fageria et al., 2005) which helps in the maintenance of soil fertility (Branca et al., 2013). Leguminous cover crops when well managed can reduce or eliminate the need for artificial N fertilizer for the succeeding cash crop (Sadeghi and Isensee, 2001; Sainju et al., 2001; Sainju et al., 2006). Several authors have documented that legume–based cropping systems reduce C and N losses from the soil (Drinkwater et al., 1998; Bronick and Lal, 2005; Sainju et al., 2006; Branca et al.,

2013). Sainju et al. (2002) observed that microbial biomass and other measures of microbial activity were increased in legume-cereal cropping systems. The use of cover crops as a short-term green manure may not necessarily enhance SOC (Sainju et al., 2003; Bronick and Lal, 2005). Species in Brassicaceae (mustard) family, with their long tap roots, have the beneficial effect of penetrating compacted soil layers to 2 m (Williams and Weil, 2004). When grown as catch crops and green manure crops, Brassicas have a significant influence on soil porosity, disease control, and weed populations (Thorup-Kristensen, 2003; Williams and Wiel, 2004; Snapp et al., 2005; Collins et al., 2007).

Adoption of cover crops in no-tillage systems results in the greatest increase in SOC (Frye et al., 1999). The increase in SOC leads to improvement in the soil structure and better soil water retention (Whitebeard et al., 2000). When cover crop residues serve as a mulch in a cropping systems, it increases microbial biomass, microbial respiration and N mineralization (Schutter and Dick, 2002). Cover crops also causes shifts in the microbial community structure thereby affecting the biological, chemical and physical properties of the soil (Schutter and Dick, 2002; Sainju et al., 2003). Additionally, cover crop residues on soil surface reduce the rate of evapotranspiration from the soil, this provides a conducive environment for increased microbial activity.

In spite of all the numerous benefits of cover crops, there is insufficient documentation on its impact on soil health in Kansas and Ghana. Additionally, there is no minimum data set for biological indicators of soil health (Bhardwaj et al., 2011). Results from this study will be incorporated into the available data for assessing chemical and physical indicators of soil health. This study will evaluate overall changes in soil improvement resulting from different management practices over time. The objectives of this research were: 1) to evaluate the impact

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of cover crops on soil health and soil microbial ecology in Kansas; and 2) to evaluate the impact of direct seeding mulch–based cropping systems (DMC) on soil health in the Guinea Savanna Zones of Ghana.

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Chapter 2 - The Impact of Cover Crops on Soil Health and Soil Microbial Ecology in Eastern Kansas

Abstract

Sustainable soil intensification practices such as use of cover crops can maintain soil health and increase crop productivity. This study was conducted to assess the effects of cover crops on soil health and microbial ecology using selected soil health indicators. Soil samples were collected (0-5 and 5-15 cm) from Ashland Bottoms, Kansas, prior to planting of sorghum, from treatments consisting of chemical fallow (CF), double-crop soybean (DCSB), summer non-legume (SNL) (sorghum-sudan grass), summer legume (SL) (late-maturing soybean), winter non-legume (WNL) (tillage radish) and winter legume (WL) (crimson clover) that received 0 and 90 kg N ha^{-1}, applied as 28% urea-ammonium nitrate. The experimental design was a split plot in randomized complete block design. Key indicators analyzed included dissolved organic C (DOC), microbial biomass C and N (MBC & MBN), potentially mineralizable N (PMN) and soil microbial respiration (SMR), bulk density (pb), water stable aggregates (WSA), microbial community structure (PLFA), and soil organic C (SOC) and total N (TN). The increase in SOC at 0-5 cm was due to addition of cover crop residue. Summer non-legume (SNL) and WNL had significantly higher rates of SMR in the top 5 cm of soil compared to CF. While DCSB, WNL, SL, and SNL also had significantly increased MBN in the top 5 cm compared to CF. Higher SMR and MBN was due to availability of crop residues. Summer cover crops significantly increased the F:B ratio. Higher F:B ratio correlates with improved SOC and aggregate stability. Changes in microbial community structure were not consistent except for actinomycetes which

were higher with DCSB and CF. In this study cover crops did not increase microbial biomass C

and had no impact on soil aggregation

Introduction

The Great Plains remains the largest contributor to the world's food supply due to its fertile soil (Liebig et al., 2004). Agricultural intensification in the Great Plains has brought significant gains in the economic and social sectors. Meanwhile it has led to land degradation and depletion of available natural resources. Conventional agricultural management practices such as tillage, conversion of grassland to pasture and arable lands, as well as continuous row cropping have negatively affected biological, chemical, and physical properties of soils in the Great Plains (Liebig et al., 2004; Collins et al., 2012). Continuous mechanical disturbance of the soil due to tillage have changed soil water dynamics, temperature, aeration, and the location of crop residues (Dick, 1992; Buckley and Schmidt, 2001; Kladivko, 2001). As such, loss of soil aggregation and structure have ramifications leading to increased surface runoff and loss of nutrients and soil (Collins et al., 2012). The loss of macroaggregates alters the microhabitat for microorganisms resulting in changes in diversity and activity (Ranjard and Richaume, 2001; Six et al., 2002). Furthermore, tillage decreases soil organic C (Fry et al., 1999; Balesdent et. al., 2000) an important energy source for soil microorganisms.

The adoption of conservation agriculture (CA) practices (i.e. No-tillage and cover crops) can reverse the effects of tillage intense systems that are associated with environmental and soil degradation (Sapkota et al., 2011). Conservation agriculture maintains organic material on the soil surface which improves the soil physical properties, nutrients, and microbial biomass and activity (Feng et al., 2003). As a result, CA improves both soil and water quality by enhancing higher soil productivity and minimizing the effects of soil erosion and water runoff (Franzleubbers, 2008). Cover crops in CA systems improve soil health by increasing microbial

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diversity, biomass, and activity (Schutter and Dick, 2002; Bronick and Lal, 2005). The change in microbial properties changes both physical and chemical properties of the soil (Schutter and Dick, 2002; Lal, 2004; Liebig et al., 2004; Bronick and Lal, 2005). Cover crops improve soil aggregation, water retention, SOC and nutrient cycling (Drinkwater et al., 1998; Lal, 2004; Liebig et al., 2004; Sainju et al., 2006). Furthermore, when legumes are used as cover crops they add N to the system through biological N fixation (Fageria et al., 2005) thus stabilizing crop yields in N-limited systems (Lal, 2004; Sainju et al., 2006).

Although there are numerous studies on the benefits of including cover crops in crop rotation systems (Liebig et al., 2004; Sainju et al., 2006; Abdollahi and Munkholm, 2014) information on biological indicators of soil health are limited. The development of biological indicators needs to be evaluated in integrated no-tillage cover crop systems for use in soil health assessment (Bhardwaj et al., 2011; Lehman et al., 2015). The objective of this study was to determine the impact of different covers crop on soil health. The specific objectives were to evaluate the effect of different cover crops on (1) microbial biomass and microbial community structure, and (2) soil structure and aggregate stability. We hypothesized that cover crops would increase fungi biomass relative to bacteria biomass, positively affecting nutrient status and increase soil aggregation.

Materials and Methods

Experimental Site

The field study was conducted at Kansas State University's Agronomy Department Research Farm located in Ashland Bottoms, about 16 km from Manhattan, Kansas, (39^009N, 96^036W). The trial was established in 2009 on a Wymore silty clay loam soil (fine smectitic, mesic Aquertic Argiudoll).

The cover crops were evaluated in a three year no–till crop rotation with a cropping sequence of winter wheat - cover crop - grain sorghum - soybean. The experimental design was a two-way factorial in a split plot in a randomized complete block design with four replications. Each main block measured 36 m x 68 m and the rotation crop was randomized within each block. Each block was separated by the cropping system, with all crop phases of the cropping systems present in each block every year. Six treatments were established between wheat harvest and sorghum planting with each plot measuring 6 m x 68 m. The cover crops were classified as legume-based covers (double-crop soybean, late-maturing soybean, crimson clover) and non-legume based covers (sorghum-sudan grass, tillage radish). Two conventional practice treatments were included. Chemical fallow (CF) treatment serves as a control was sprayed at least twice between wheat harvest and frost to control weeds and volunteer wheat. A double-crop soybean treatment was planted where the summer cover crop treatments were planted and harvested for grain. Cover crop treatments were established based on the season that they were grown, either in summer or winter. During summer, late-maturing soybean (*Glycine max*) and sorghum-sudan grass (*Sorghum vulgar var.sudanese*) were established as summer legume (SL) and summer non-legume (SNL), respectively. Both the SL and SNL were planted after wheat harvest and terminated in mid Septembers with a roller-crimper at early pod set and early head stages,

respectively. During the late summer, crimson clover (*Trifolium incarnatum* L) and tillage radish (*Brassica*) were established as winter legume (WL) and winter non-legume (WNL), respectively. Both were planted in mid–late August and terminated by frost or chemical methods in spring. The WL was chemically terminated in the 3rd week of April prior to planting of sorghum. The WNL was terminated by both freezing temperatures and herbicides during winter to control weeds and volunteer wheat. In 2014, sorghum was planted on all plots. Generally, weed control among the cover crops was done by applying 1-2 herbicide applications.

With regards to the sub plot factor, each cover crop plot was divided into five subplots of dimension 6 m x 14 m per plot, and five different N fertilizer rates (0, 45, 90, 135, and 180 kg N ha^{-1}) were applied from Urea Ammonium Nitrate (UAN) during the sorghum phase. For the purposes of this study, we restricted our soil sampling to cover crops treatments that received 0 and 90 kg N ha^{-1}, respectively.

Soil Sampling

Soil samples were collected in May 2014 from treatments previously planted to cover crops prior to planting to sorghum. Twelve soil cores were randomly collected at depths of 0-5 and 5-15 cm across each plot using soil probes of 1.9 cm diameter. The field moist soil samples were then pooled, mixed thoroughly, and passed through a 6-mm mesh screen to remove large plant material and soil fauna. The processed soil samples were stored in plastic zip lock bags at 4 C^o until further analysis. Soil water content for each soil sample was determined and the soil water content adjusted to 0.28 kg kg^{-1} which was equivalent to 60% water filled pore space (WFPS) before microbial analysis. When the gravimetric soil water content was < 0.28 kg kg^{-1}, water was added to adjust it back to this level.

Soil Microbial Biomass Carbon and Nitrogen (MBC and MBN)

Microbial biomass C and N were determined using the fumigation-incubation method (Jenkinson and Powlson, 1976). Field moist soil (25g) each was weighed into two 125 mL Erlenmeyer flasks. One set of flasks were fumigated with chloroform in a vacuum desiccator containing wet paper towels and a beaker with 70 mL of ethanol-free chloroform with a boiling chip. The desiccator was sealed with vacuum grease and placed under laboratory hood, and evacuated three times for ~ 2 min each round of evacuation, to allow the chloroform to boil. After the third evacuation, the desiccator was tightly closed to allow the chloroform to diffuse into the soil. Both the fumigated and the non-fumigated samples were kept in the dark. After 20 h, the beaker and the paper towels were removed and the desiccator was evacuated 10 times for 3 min each time. Both samples were placed in 940 mL mason jars containing enough (50mL) water to maintain a humidified atmosphere. The mason jars were tightly closed and incubated for 10 days at 25°C. After 10 days of incubation, soil microbial respiration from both fumigated and the non- fumigated samples were determined by sampling the headspace for the evolved CO_2-C and analyzed using a gas chromatograph (Shimadzu GC-8A, Shimadzu Scientific Instruments, and Columbia, MD) (Gajda and Martyniuk, 2005). MBC was calculated as the difference between evolved CO_2 from fumigated soil and non-fumigated soil and divided by a conversion factor - $K_C = 0.45$ (fraction of biomass C mineralized into to CO_2) (Jenkinson and Powlson, 1976). Likewise, MBN was determined by adding 100 mL of 1 M KCl to both fumigated soil and non-fumigated soil and shaken for 1 h on an orbital shaker at 300 rpm. Suspensions were filtered through Whatman filter paper No. 42 into 20 mL scintillation vial and analyzed colorimetrically for inorganic N (NH_4-N and NO_3-N) (Gelderman and Beegle, 1998; Mikha and Rice, 2004; Maynard et al., 2006) at KSU Soil Testing Lab. MBN was then calculated as the

difference between inorganic N evolved from fumigated and non-fumigated soil and divided by a conversion factor of $K_C = 0.54$ (Jenkinson and Powlson, 1976).

Soil Inorganic N

Soil inorganic N was extracted by adding 100 mL of $1M$ KCl to 25g of field moist soil sample. The soil samples were then shaken for 60 min on an orbital shaker at 300 rpm, and filtered through Whatman No. 42 filter paper into a 20 mL scintillation vial. The extract was analyzed for NH_4-N and NO_3-N using a colorimetric analysis (Gelderman and Beegle, 1998; Mikha and Rice, 2004; Maynard et al., 2006) at KSU Soil Testing Lab.

Potentially Mineralizable N

Potentially mineralizable nitrogen (PMN) was determined as the difference between inorganic N (NH_4-N and NO_3-N) in field moist soil sample and inorganic N in the non-fumigated (NH_4-N and NO_3-N) incubated soil samples (Maynard et al., 2006; Gugino et al., 2009).

Soil Bulk Density

Soil probes with inner diameter of 1.9 cm were used to randomly take nine cores per experimental unit from two different depths, 0-5 cm and 5-15 cm. The soil samples were put in plastic zip lock bags and transported to the laboratory. The field moist soil samples were weighed and oven dried at 105^0C for 48 h and then re-weighed. The bulk density was calculated from the weight of the oven dried soil and soil volume (Hao et al., 2006).

Water Stable Aggregates

Two undisturbed soil samples were collected using a spade from each cover crop treatment at 0-5 and 5-15 cm depths for soil aggregate stability. The soil samples were placed in plastic zip lock bags and stored at 4^oC. Soil aggregate stability was assessed according to the method by Tripathi et al. (2014) with little modification. Briefly the field moist soils were air

dried for 96 h, crumbled and then passed through a 4-mm sieve. Soil aggregates retained on the 4-mm sieve were separated by wet sieving (Camberdella and Elliott, 1992). Two 50g air dried soil samples were placed on a nest of sieves with 1000, 250, 53 and 20 µm openings to obtained four size fraction of >1000, 1000-250, 250-53, 53-20 µm from each treatment. Two stacked sieves of 1000 and 250 µm were placed into a bucket and connected to a motor. Deionized water (3L) was added to the bucket to bring the water level to the base of the top sieve. The soil samples were immerse for 10 min. Thereafter the sieves were raised and lowered 4 cm at 30 oscillations per minute for 10 min. Floating organic material were decanted and aggregates retained on the two sieves were transferred into aluminum pan. After wet sieving, soil plus the remaining water in the bucket was poured onto the finer sieves (250-53 and 53-20 µm mesh). The two sieves were stacked together and shaken for 3 min, to allow water and particle fractions smaller than the sieve size to pass through. Soils retained on each sieve were backwashed into an aluminum pan. Aggregates <20 µm were discarded and soil recovery was calculated. Aggregate fractions for total C and N analysis were oven dried at 65°C for 48 h (Mikha and Rice, 2004). For water stable aggregate (WSA) determination the samples were oven dried 105°C for 48 h and weighed. Sand free WSA was measured by weighing a subsample (2–5 g) of separated aggregates fraction and combining it with 5 times the volume (10-25 mL) of 5 g L^{-1} of sodium hexametaphosphate. Samples were left overnight and shaken on an orbital shaker at 325 rpm for 5 h. The dispersed organic matter and sand was collected on a 53 µm mesh sieve, washed with deionized water, and dried at 105°C for 24 h, and the sand fraction weighed.

The water stable aggregate was computed as

$$\text{WSA (g kg}^{-1}) = \frac{(\text{Weight of soil retained}) - (\text{Weight of sand})}{(\text{Total soil weight}) - (\text{Weight of sand})} \times 1/1000$$

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Soil Organic Carbon (SOC) and Total Nitrogen (TN)

Air-dried soil samples were ground to a fine powder using a mortar and pestle after removing the roots. The grounded soil samples were sieved through a 53 µm mesh sieve. Soil samples were then analyzed for SOC and TN content by dry combustion using a C N Analyzer (EA 112) (Mikha and Rice, 2004).

Dissolve Organic Carbon (DOC)

Field moist soil was passed through a 2 mm mesh sieve to separate roots and organic debris. Soil (8g) was placed in an acid washed 125 mL Erlenmeyer flasks with 40 mL of 0.5 M K_2SO_4 (Ghimire et al., 2014). The samples were then shaken for 30 min on an orbital shaker at 300 rpm, and filtered through Whatman No. 42 filter paper into 60 mL acid washed HDPE vials (Clear VOA Glass Vials). The extracted samples were then stored at -10°C. The samples were analyzed using TOC Shimadzu analyzer (Shimadzu Corporation, Japan) (Magill and Abe, 2000).

Phospholipid fatty acid (PLFA)

Phospholipid fatty acid (PLFA) extraction was determined according to a modified (Bligh and Dyer, 1959; White and Ringelberg, 1998) method. Prior to analysis, all glassware was placed in a muffle furnace at 400°C for 5 h and forceps and working area were cleaned with acetone to avoid contamination of samples. Approximately 5 g of freeze-dried soil was suspended in a 2:1:0.8 extraction solution which contained methanol, chloroform and phosphate buffer, and vortex briefly. The suspension was allowed to stand for 3 h within which it was briefly vortex every hour and centrifuge at 1500 rpm for 10 min. After centrifugation, the supernatant was transferred into 50 mL test tubes with 5 mL chloroform and 5 mL nanopure water and vortex for 1 min and left overnight in a darkroom at 25°C. After overnight separation, the organic phase (lower phase) was transferred into another test tube and dried using a rotary evaporator (N-EVAP) at 50°C. The

resulting dried portion was re-dissolved in chloroform and using a preconditioned disposable silica gel extraction columns (J.T. Baker, Phillipsburg, NJ, USA), was separated into three fractions: neutral lipid, glycolipid, and polar lipid (Zelles and Bai, 1993). The methanol extracts containing the polar lipid fractions (PLFA) was dried using a rotary evaporator under nitrogen. The polar lipid fraction (PLF) were saponified and methylated to fatty acid methyl esters (FAME) (White et al., 1979). An internal standard prepared from methyl non deaconate fatty acid was added to FAMEs and stored at −18 °C until analysis. FAMEs were analyzed using Thermo Scientific Trace GC-ISQ mass spectrometer (Thermos Scientific, USA) with a DB5-MS column using helium as carrier gas. Peaks were recognized by matching retention times with known standards (Bacterial Acid Methyl Ester Mix-CP, Supelco 47080; Sigma Aldrich) based on the International Union of Pure and Applied Chemistry system (IUPAC). The concentration of each PLFA was obtained by quantifying peak areas with a 19:0 standard curve. Standard nomenclature was used to describe the PLFAs. Specific PLFA signatures were used to quantify the relative abundance of various taxonomic groups: (1) i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0 for gram-positive bacteria; (2) cy17:0, 16:1 ω 9c, 17:1 ω 9c, and 18:1 ω 7c, and cy19:0 for gram-negative bacteria; (3) 18:2 ω 6c for fungi; and (4) actinomycetes for 10Me16:0, 10Me17:0, and 10Me18:0). The total bacterial biomass was calculated as the sum of i15:0, a15:0, i16:0, 16:1 ω 9, 16:1 ω 5, and 17:1 ω 9, i17:0, a17:0, cy17:0, 18:1 ω 7, and cy19:0 PLFAs (Frostegard and Ba°a°th, 1996; Blume et al., 2002).

Data Analysis

Data analysis was done using Proc mixed model in SAS 9.4 (SAS institute, Inc. 2014).

Treatments means were separated using Fisher protected least significant difference (LSD).at

significance level of $p = 0.10$

Results and Discussion

Soil Physical Indicators

Soil bulk density (pb)

Bulk density was not significantly (P < 0.10) affected by cover crops and N fertilizer irrespective of depth (Table 2.2 and Table 2.3). Bulk density ranged from 1.2 to 1.3 g cm^{-3} and 1.3 to1.4 g cm^{-3} at 0-5 and 5-15 cm, respectively. These results fall within the acceptable range of <1.6 g cm^{-3} for plant production (USDA-NRCS, 2003; Karlen et al., 2008; Sapkota et al., 2011). Bulk density is known to affect soil microbial activity, infiltration, available water holding capacity, soil porosity, rooting depth /restrictions and plant nutrient availability, which influence key soil processes and productivity (USDA-NRCS, 2003).

Water Stable Aggregates (WSA)

Aggregate structure for macroaggregates was not significantly (P < 0.10) affected by the cover crops and N fertilizer irrespective of depth treatments (Table 2.2). Abdollahi and Munkholm (2014) also reported that cover crops did not affect WSA. In general macroaggregates (>1000-250 μm) were greater with the cover crops and then chemical fallow at the 0-5 cm depth. Winter non legume (WNL) had the greatest mean for water stable macroaggregate (396 g kg^{-1}) while CF had the least (265 g kg^{-1}). Meanwhile cover crop had a significant effect (P<0.10) on microaggregates (250-20 μm) at the 0-5cm depth. Winter legume (WL) had higher water stable microaggregates (600 g kg^{-1}) compared to WNL (506 g kg^{-1}) and DCSB (519 g kg^{-1}) at the 0-5cm depth (Table 2.2). Nitrogen fertilizer application did not significantly affect aggregate distribution at the different depths.

The interactive effect of cover crops and mineral fertilizer was significant (P < 0.10) for the macroaggregates at the 5-15 cm depth (Table 2.3). However the greatest differences was seen

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between the N rates with winter non legume (WNL). The greater microaggregates under WL (crimson clover) suggest poor soil structure development. Since aggregate stability depends on binding agents such as microbial and plant polysaccharides (Six et al., 2004), the high bacteria and fungi biomass found at the surface 0-5 cm soil probably played a key role in soil aggregation. Similarly, these binding agents possibly explain the greater magnitude of water stable macroaggregates in the 0-5 cm than 5-15 cm depths. The availability of substrate provided the needed labile C source for increase microbial activity and population in the top soil. Macroaggregates are held by other organic C as well as the biological agents (Tindall and Oades, 1982; Oades, 1993; Six et al., 2004; Deb et al., 2015).

Soil Chemical Indicators

Soil Organic Carbon (SOC)

Soil organic C was not significantly different with respect to cover crops regardless of depth. Soil organic C was by 5 to 22 % higher in the cover crop systems relative to the chemical fallow at 0-5 cm and 8% higher at 5-15 cm (Table 2.4). This infers the cover crops in the study have the potential to increase SOC. Several authors have reported that organic inputs is necessary to increase SOC (Lal, 2004; Deb et al., 2015). Addition of N fertilizer increased SOC by 5% in the top 0-5 cm and was significantly ($P < 0.1$) decreased by 8% in the 5-15 cm depth (Table 2.4). Combining cover crops with fertilizer did not significantly affect SOC at both depths (Table 2.5).

Although, SOC was not significant, some form of residue management may be necessary to enhance SOC (Schoenholtz et al., 2000; Lal, 2004). Our results show that all cover crops resulted in a numeric increase in SOC compared to CF. The numerical increased in SOC in the top 0-5 cm due to N fertilizer suggests that mineral fertilizer especially N may be needed to

balance the C:N ratio of the added plant material (Gregorich et al., 1994; Paustian et al., 1997; Giller, 2002; Dick and Gregorich, 2004).

Total Nitrogen (TN)

Irrespective of depth, TN was not significantly ($P < 0.10$) affected by cover crops (Table 2.4). At 0-5 cm, the difference between the highest and lowest TN by cover crop was 8 %. Application of mineral fertilizer did not significantly change TN in the top 0-5 cm (Table 2.4). In the sub-surface layer (5-15 cm) N application significantly ($P < 0.10$) reduced TN by 8 %. The interactive effect of cover crops and fertilizer was not significant for TN at both depths (Table 2.5). The trend of TN under cover crops was similar to SOC. Conservation of SOC results in conservation of TN (Drinkwater et al., 1998; Bronick and Lal, 2005; Sainju et al., 2006; Branca et al., 2013).

Aggregate Associated SOC and TN

Aggregate associated SOC and TN in both macroaggregates (>1000 µm and 1000-250 µm) and microaggregates (250-53 µm and 53-20 µm) were not significantly affected by cover crops and fertilizer (Table 2.6 and Table 2.8). Generally, higher concentration of SOC and TN were associated with the macroaggregates compared to the microaggregates irrespective of depth (Table 2.6 and Table 2.8). This confirms the assertion made by Mikha and Rice (2004) that macroaggregates contain higher concentrations of C and N than microaggregates. The addition of organic residue supports macroaggregate formation and the associated C and N (Lal, 2004; Six et al., 2004; Deb et al., 2015). At 5-15 cm depth, SOC and TN associated with macroaggregates 1000-250 µm was significantly affected by the interaction of cover crops and N fertilizer as DSB + 90 N kg ha^{-1} and WL+ 90 N kg ha^{-1} had greater SOC compared to CF +90 N kg ha^{-1} (Table 2.7 and Table 2.9).

Dissolved Organic Carbon (DOC)

Dissolved organic carbon is also another labile C pool derived from plant roots, leaf litter, and humus that serve as substrate for microbial activity (Liu et al., 2013). DOC was not significantly (P< 0.1) affected by cover crops and N fertilizer at both 0-5 cm and 5-15 cm depths (Table 2.10). WNL had the highest DOC of 70 mg kg^{-1} while CF had the lowest DOC of 61 mg kg^{-1} at 0-5 cm depth (Table 2.10). At 5-15 cm, SL had greater DOC of 59 mg kg^{-1} compared to DCSB with the lowest DOC of 52 mg kg^{-1} (Table 2.10). This represents about 13% increase in labile C over DCSB. N fertilizer alone and the interactive effect of cover crops and N had no significant effect on DOC at both the 0-5 cm and 5-15 cm (Table 2.11).

In general higher concentrations of DOC were found in the surface 5 cm layer relative to the 5-15 cm layer which could be attributable to the presence of higher organic matter in the surface layer (Table 2.1) (Liu et al., 2013). The lowest DOC under CF was expected because it retains the least amount of residue. The presence of high DOC under WNL was likely affected by the increased microbial activity (respiration) and this influence macroaggregate associated SOC at the 0-5 cm depth. This confirms the assertion that DOC is a labile pool that serve as substrate for microorganisms (Liu et al., 2013; Ghimire et al., 2014).

Soil Biological Indicators

Microbial Biomass Carbon (MBC)

Microbial biomass C was not significantly (P < 0.10) affected by treatments at both depths. Although not significant MBC was higher in DCSB (Table 2.10). DCSB potentially contributes more labile C to the soil organic pool.

The higher MBC under the DCSB could be due to the narrow C:N of residue favoring microbial growth (Balota et al., 2003). The MBC as a soil health indicator depends on the addition of labile C and favorable environmental conditions (moisture, temperature etc.). Thus MBC could be a potential indicator for assessing changes in soil quality because it can either be a sink or source of plant available nutrients (Balota et al., 2011).

Microbial Biomass Nitrogen (MBN)

At 0-5 cm, MBN was significantly $(P < 0.10)$ affected the cover crops. Double–crop soybean had greater increased in MBN compared to the chemical fallow (CF). In general cover crops have higher MBN compared to CF (Table 2.10). This is probably due to high turnover of cover crop biomass. MBN was significantly $(P < 0.10)$ increased by the interactive effect of cover crops and N fertilizer in the 5-15 cm layer. SNL + 90 N kg ha^{-1} had the most increased MBN (Table 2.11). The effect due to interaction suggests that some cover crops will require N fertilizer to enhanced high MBN pool.

Soil Microbial Respiration (SMR)

Soil microbial respiration was not significantly affected by cover crops and N fertilizer at the 0-5 cm depth (Table 2.10). At the 5-15 cm depth, soil microbial respiration was significantly $(P < 0.10)$ greater in SNL (88 μg C g^{-1} soil 10 day^{-1}) and least in SL (76 μg C g^{-1} soil 10 day^{-1}) and DCSB (77 μg C g^{-1} soil 10 day^{-1}) (Table 2.10). The interaction of cover crops and N fertilizer did not significantly affect soil respiration regardless of depth (Table 2.11).

Potentially Mineralizable Nitrogen (PMN)

Potentially mineralizable N (PMN) was not significantly affected by the treatments (Table 2.10). Although not significant, WL had greater PMN at the 0-5 cm depth. This may be

due to the plant residue N available for mineralization. The increase in PMN was about 83% compared to CF at the 0-5 cm depth. The application of N fertilizer increased PMN by 8% in the 0-5 cm depth, indicating the value of added plant organic N to the system. Sharifi et al. (2007) also observed high PMN on soil with crop residue.

Soil Microbial Community Structure

Actinomycetes

The soil microbial community structure was assessed using phospholipid fatty acid analysis (PLFA). The PFLA provides information on changes within the microbial community. Actinomycetes were significantly affected by cover crop in the 0-5 cm depth as more actinomycetes were found in CF and DCSB compared to the SNL, WNL and WL treatments (Table 2.12). The interactive effects of cover crop and N fertilizer was significant in the 5-15 cm depth. SL + 0 N kg ha^{-1}, SNL + 90 N kg ha^{-1}, WL+ 90 N kg ha^{-1} and DCSB + 0 N kg ha^{-1} had more actinomycetes compared to WNL + 0 N kg ha^{-1} (Table 2.13). The interaction effect indicates that N fertilizer added to some cover crops increases actinomycetes population. Martyniuk and Wagner (1978) found more actinomycetes in a corn, oat, wheat and red clover rotation compared to continuous cropping of maize or wheat. Therefore, the rotational crop system could be the reason for the increased actinomycetes. Furthermore, Dung et al. (2010) found diversified actinomycetes community in corn – rice- mung bean crop rotation system in Vietnam.

Gram negative (gram–ve) bacteria

Gram negative bacteria were not significantly affected by the different cover crops (Table 2.12). Application of N fertilizer did not significantly affect gram-ve bacteria (Table 2.12). The

interactive effect of cover crops and N fertilizer had no significant effect on gram-negative bacteria at both the 0-5 cm and 5-15 cm (Table 2.13).

Gram positive (gram +ve) bacteria

Gram positive bacteria at the 5-15 cm depth was not significantly affected by the interaction of cover crops and N (Table 2.13). At 5-15 cm, winter legume (WL) and DCSB had significantly ($P < 0.10$) higher gram+ve bacteria than SNL. While at the 0-5 cm, gram+ve bacteria were more abundant in the WNL compared to the other cover crop systems though not significant (Table 2.12).

Saprophytic fungi and arbuscular mycorrhizae fungi (AMF)

Irrespective of depth, both saprophytic fungi and arbuscular mycorrhizae fungi (AMF) were not statistically significant with regards to cover crops and N fertilizer (Table 2.12 and Table 2.13).

Fungi:Bacteria Ratio

Fungi:Bacteria (F:B) ratio informs about the dominance of fungi to bacteria in ecosystems due to the effects of crop and soil management. A significantly ($p < 0.1$) higher fungi:bacteria ratio was observed for SL compared to the other cover crop systems at 0-5 cm (Table 2.14). The interactive effect of cover crop and N fertilizer significantly affected the F:B ratio at 0-5 cm and 5-15 cm depths (Table 2.15). At the 0-5 cm, SL + 90N kg ha^{-1} had a greater increased in F:B ratio compared to the other treatments At the 5-15 cm depth, SNL+ 80 N kg ha^{-1} had significantly higher F:B ratio compared to WNL + 90 N kg ha^{-1}, WL+ 90 N kg ha^{-1}, and CF + 90 N kg ha^{-1} (Table 2.15). A higher F:B ratio as observed in SNL (sorghum) due interaction is probably due to increase below ground biomass (root) with high-C based substrate (Ghimire et al., 2014).

Agricultural systems with fungal dominance have higher soil C sequestration potential (Busse et al., 2009). Higher F:B ratio is often used to predict SOC sequestration potential as well as soil aggregate stability (Busse et al., 2009; García-Orenes et al, 2013; Ghimire et al., 2014). High F:B ratio seen under these cover crops did not translate into a significant increased SOC and aggregate stability. The SL treatment also had higher macroaggregates. Fungi play a key role in the formation of macroaggregates (Tisdall and Odes, 1982; Helgason et al., 2014).

Total PLFA

The interactive effect of cover crops and N fertilizer was significant ($P < 0.1$) in the 5-15 cm, as CF + 0 N kg ha $^{-1}$ had the highest total PLFA biomass compared to the other treatments except WL + 0 N kg ha^{-1}, WL+ 90 N kg ha^{-1} (Table 2.15). This may be due to substrate availability for microorganisms.

Conclusion

Knowing how soil properties, particularly soil biological indicators of health, respond to management is needed to accurately assess soil health. Several authors have reported that cover crops can improve soil health over time. Results from our study showed that cover crops did not increased microbial biomass, and aggregate stability which is in contrast to our hypothesis. However, cover crops affected MBN, SMR and microbial community structure (F:B ratio, actinomycetes, and total PLFA). These changes may be due to increased plant residues with the cover crops. WNL appears to the most effective cover crops in improving soil aggregate stability. The interactive effect appears to have affected some soil health indicators; macroaggregate formation, aggregate SOC and TN, MBN, bacteria, actinomycetes, total PLFA and F:B. Nitrogen fertilizer did not consistently affect the soil health indicators examined in this experiment. Results from this study did not show consistent effects for indicators of soil health. It is not known why we did not see the expected results. This could be due to soil type, length of the experiment or time of sampling. We recommend that assessment should be repeated since soil health indicators especially biological indicators are influenced many factors such as crop pattern, weather, moisture, and availability of substrate. Future research should focus on detail sampling to match up with weather and more biological indicator such as soil enzymes should also be assessed.

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Table 2.1. Soil chemical properties at Ashland Bottoms, Kansas.

Variable(s)	2008			2009		
	Depth (cm)					
	0-5	5-10	10-15	0-5	5-10	10-15
SOM (g kg^{-1})	110	13.0	12.8	24.0	22.0	21.5
pH	6.1	6.4	5.9	5.9	6.1	5.9
Buffer pH SMP	8.3	5.1	6.7	6.8	6.8	6.8
Mehlich-3 P (mg kg^{-1})	70.0	14.3	10.8	34.8	15.8	11.5
K (mg kg^{-1})	632	326	226	402	300	254
Zn (mg kg^{-1})	3.5	2.5	2.3	11.9	1.2	1.2

Source: Arnet (2010)

Table 2.2. Soil bulk density and water stable aggregates as affected by cover crops and mineral fertilizer at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)					
	0 – 5			5.- 15		
		Aggregate size			Aggregate size	
	Density	Marco	Micro	Density	Marco	Micro
	g cm^{-3}	(g kg^{-1})		g cm^{-3}	(g kg^{-1})	
Cover crops						
CF	1.2	265	597 ab	1.4	222	692
DCSB	1.2	356	519 bc	1.4	237	675
SL	1.2	372	529 abc	1.4	249	676
SNL	1.3	341	573 abc	1.4	261	643
WL	1.2	314	600 a	1.4	236	659
WNL	1.2	396	506 c	1.3	267	623
LSD (P<0.1)	NS	NS		NS	NS	NS
Fertilizer (kg ha^{-1})						
0 N	1.2	352	540	1.4	253	659
90 N	1.2	330	568	1.4	237	663
LSD (P<0.1)	NS	NS	NS	NS	NS	NS
	P>F				P>F	
Cover crops	0.4237	0.1049	0.093	0.5802	0.784	0.5422
Fertilizer	0.302	0.2412	0.1747	0.8248	0.4148	0.8513

Mean in the same column with the same letter(s) are not significantly different at P < 0.1.

NS = Not significantly different.

Macroaggregates = > 1000 – 250 µm
Microaggregates = 250–20 µm.
Aggregate sizes are sand –free

CF - Chemical fallow
DCSB - Double crop soybean
SL - Summer legume
SNL - Summer non-legume
WL - Winter legume
WNL – Winter non-legume

Table 2.3. Soil bulk density, water stable aggregates affected by the interaction of cover crops and mineral fertilizer at different depths at Ashland Bottom, Kansas, 2014.

	Depth(cm)					
	0-5	5 -15	0-5	5-15	0-5	5-15
	Soil bulk density		Macroaggregate		Microaggregates	
Interactive effect			>1000-250 µm		250-20 µm	
CC x Fert (kg ha^{-1})	g cm^{-3}		g kg^{-1}		g kg^{-1}	
CF + 0 N	1.2	1.4	263	292 a	578	620
CF +90 N	1.3	1.5	268	152 b	618	763
DCSB + 0 N	1.2	1.4	366	217 ab	508	681
DCSB + 90 N	1.2	1.3	347	257 a	530	670
SL + 0 N	1.2	1.4	397	282 a	512	658
SL+90 N	1.2	1.4	347	217 ab	547	693
SNL + 0 N	1.2	1.4	316	225 ab	606	683
SNL+90 N	1.3	1.4	366	297 a	539	603
WL + 0 N	1.3	1.4	351	213 ab	555	603
WL+ 90 N	1.2	1.4	276	260 a	645	638
WNL + 0 N	1.2	1.3	419	299 a	481	633
WNL + 90 N	1.1	1.4	374	241 ab	531	613
LSD (P<0.1)	NS	NS	NS		NS	NS
	P>F					
CC x Fertilizer	0.4093	0.1688	0.4573	0.0281	0.3844	0.1089

Means in the column with same letter(s) are not significant.
NS =not significant different.
CC = Cover crop.　　　Fert = Fertilizer.
For abbreviations on cover crop see Table 2.2.

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Table 2.4. Soil organic carbon (SOC) and total N (TN) as affected by cover crop and mineral fertilizer at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)			
	0 - 5	5 - 15	0 – 5	5 - 15
	SOC		TN	
	g C kg^{-1}		g N kg^{-1}	
Cover crop				
CF	18	12	1.7	1.3
DCSB	20	13	1.9	1.3
SL	20	13	1.9	1.3
SNL	22	13	2.0	1.3
WL	19	13	1.8	1.3
WNL	19	13	1.8	1.4
LSD (P<0.1)	NS	NS	NS	NS
Fertilizer (kg ha^{1})				
0 N	19	13 a	1.8	1.3 a
90 N	20	12 b	1.8	1.2 b
LSD (P<0.1)	NS		NS	
	P>F		P>F	
Cover crop	0.2449	0.2694	0.2663	0.1954
Fertilizer	0.9517	0.0735	0.9822	0.0875

Mean in the same column with the same letter(s) are not significantly different at P < 0.1.
NS = Not significantly different.

For abbreviations on cover crop see Table 2.

Table 2.5. Soil organic carbon (SOC) and total N (TN) as affected by the interactive effect of cover crop and mineral fertilizer at different depths at Ashland Bottom, Kansas, 2014.

Interactive effect	Depth (cm)			
	0-5	5-15	0-5	5 -15
	SOC		TN	
	g C kg^{-1}		g N kg^{-1}	
CC x Fertilizer (kg ha^{-1})				
CF + 0 N	17	13	1.7	1.3
CF +90 N	18	12	1.7	1.2
DSB + 0 N	20	13	1.9	1.3
DSB + 90 N	20	13	1.9	1.3
SL + 0 N	20	13	1.9	1.4
SL+90 N	19	12	1.8	1.2
SNL + 0 N	22	13	2.0	1.3
SNL+90 N	22	13	2.0	1.3
WL + 0 N	18	12	1.7	1.3
WL+ 90 N	19	13	1.8	1.3
WNL + 0 N	19	13	1.8	1.4
WNL + 90 N	19	13	1.8	1.4
LSD (P<0.1)	NS	NS	NS	NS
	P > F		P > F	
CC X Fertilizer	0.8705	0.1223	0.8328	0.1132

NS = Not significantly different.

CC = Cover crop.

For abbreviations on cover crops see Table 2.2.

Table 2.6. Soil organic C (SOC) as affected by cover crops and mineral fertilizer on aggregate-size classes at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)							
	0 – 5				5 - 15			
	Aggregate size classes (µm)				Aggregate size classes (µm)			
	>1000	1000-250	250-53	53-20	>1000	1000-250	250-53	53-20
	Aggregate SOC (g C kg^{-1})							
Cover crop								
CF	36	23	13	11	20	14	11	10
DCSB	35	24	15	11	22	15	11	11
SL	37	24	16	9	24	15	10	10
SNL	37	26	14	10	21	15	11	10
WL	32	22	13	10	21	15	11	11
WNL	38	23	14	11	20	15	11	10
LSD (P<0.1)	NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer (kg ha^{-1})	>1000	1000-250	250-53	53-20	>1000	1000-250	250-53	53-20
0 N	38	24	14	11	22	15	11	10
90 N	34	23	14	11	22	15	11	10
LSD (P<0.1)	NS	NS	NS	NS	NS	NS	NS	NS
	P > F				P >F			
Cover crop	0.85	0.194	0.412	0.252	0.278	0.109	0.351	0.311
Fertilizer (kg ha^{-1})	0.142	0.990	0.234	0.981	0.776	0.278	0.141	0.415

NS = Not significantly different.
For abbreviation on cover crops see Table 2.2.

Table 2.7. Soil organic C (SOC) as affected by the interaction of cover crops and mineral fertilizer on aggregate classes at different depths at Ashland Bottom, Kansas, 2014.

	Macroaggregate Classes				Microaggregate Classes			
	>1000 µm		1000-250 µm		250-53 µm		53-20 µm	
	Depth (cm)							
Interactive effect	0-5	5-15	0-5	5-15	0-5	5-15	0-5	5-15
CC X Fert (kg ha^{-1})	Aggregate SOC (g C kg^{-1})							
CF + 0 N	35	22	24	15 ab	14	11	11	10
CF +90 N	36	19	22	14 b	13	10	10	9.6
DCSB + 0 N	36	23	24	15 ab	17	11	11	10
DCSB + 90 N	35	20	24	16 a	14	11	11	11
SL + 0 N	42	23	26	15 ab	15	11	10	10
SL+90 N	32	26	23	15 ab	16	10	8.4	10
SNL + 0 N	38	22	26	15 ab	14	11	10	9.9
SNL+90 N	35	21	25	15 ab	14	11	10	11
WL + 0 N	33	20	21	15 ab	13	11	11	11
WL+ 90 N	32	22	22	16 a	14	11	9.8	11
WNL + 0 N	43	20	23	15ab	14	11	11	10
WNL + 90 N	34	20	23	15 ab	14	12	11	10
LSD (P<0.1)	NS	NS	NS		NS	NS	NS	NS
	P > F				P > F			
CC X Fertilizer	0.681	0.156	0.681	0.052	0.658	0.100	0.658	0.186

Means in the column with same letter(s) are not significant at P < 0.1. NS =not significant different.

CC = Cover crop.　　　Fert = Fertilizer.

For abbreviations on cover crops see Table 2.2.

Table 2.8. Soil total Nitrogen (TN) as affected by cover crops and mineral fertilizer on aggregate size at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)							
	0–5				5-15			
	Aggregate size classes (µm)				Aggregate size classes (µm)			
	> 1000	1000-250	250-53	53-20	>1000	1000-250	250-53	53-20
	Aggregate TN (g N kg^{-1})							
Cover crop								
CF	3.0	2.2	1.4	1.3	2.0	1.5	1.1 b	1.1
DCSB	3.0	2.3	1.6	1.3	2.1	1.5	1.2 a	1.1
SL	3.1	2.3	1.6	1.1	2.3	1.5	1.1 b	1.1
SNL	3.1	2.4	1.5	1.2	2.0	1.5	1.2 a	1.1
WL	2.8	2.1	1.4	1.3	2.1	1.5	1.2 a	1.2
WNL	3.1	2.2	1.5	1.3	2.0	1.5	1.2 a	1.1
LSD (P<0.1)	NS	NS	NS	NS	NS	NS		NS
Fertilizer (kg ha^{-1})	>1000	1000-250	250-53	53-20	> 1000	1000-250	250-53	53-20
0 N	3.1	2.3	1.5	1.3	2.1	1.5	1.2	1.1
90 N	2.9	2.2	1.5	1.2	2.1	1.5	1.2	1.1
LSD (P<0.1)	NS	NS	NS	NS	NS	NS	NS	NS
	P > F							
Cover crop	0.937	0.133	0.471	0.167	0.419	0.519	0.003	0.289
Fertilizer	0.138	0.671	0.438	0.794	0.951	0.558	0.277	0.968

Means in the column with same letter(s) are not significant at P < 0.1.

NS = not significantly different.

For abbreviations on cover crop see Table 2.2.

Table 2.9. Soil total Nitrogen (TN) as affected by the interaction of cover crops and mineral fertilizer on aggregate classes at different depths at Ashland Bottom, Kansas, 2014.

	Macroaggregate Classes				Microaggregate Classes			
	>1000 μm		1000-250 μm		250-53 μm		53-20 μm	
	Depth (cm)							
Interactive effect	0-5	5-15	0-5	5-15	0-5	5-15	0-5	5-15
(kg ha^{-1})	Aggregate TN (g N kg^{-1})							
CF + 0 N	2.9	2.1	2.3	1.5 ab	1.4	1.2 bc	1.3	1.1
CF +90 N	3	1.9	2.1	1.4 b	1.4	1.1 c	1.2	1.1
DCSB + 0 N	3	2.2	2.3	1.5 ab	1.7	1.2 bc	1.3	1.1
DCSB + 90 N	2.9	1.9	2.3	1.6 a	1.4	1.3 ab	1.3	1.2
SL + 0 N	3.4	2.2	2.4	1.5 ab	1.5	1.1 c	1.2	1.2
SL+90 N	2.8	2.5	2.2	1.5 ab	1.6	1.1 c	1	1.1
SNL + 0 N	3.1	2	2.4	1.5 ab	1.5	1.2 bc	1.2	1.1
SNL+90 N	3	1.9	2.3	1.5 ab	1.5	1.2 bc	1.2	1.2
WL + 0 N	2.9	2	2.1	1.5 ab	1.4	1.1 c	1.3	1.2
WL+ 90 N	2.8	2.1	2.2	1.6 a	1.5	1.1 c	1.3	1.2
WNL + 0 N	3.3	2	2.1	1.5 ab	1.4	1.1 c	1.3	1.1
WNL + 90 N	2.9	2	2.2	1.5 ab	1.5	1.5 a	1.3	1.1
LSD (P<0.1)	NS	NS	NS		NS	NS	NS	NS
	P > F				P > F			
CC X Fertilizer	0.681	0.198	0.539	0.051	0.458	0.031	0.692	0.101

Means in the column with same letter(s) are not significant at P < 0.1.   NS = Not significantly different.

CC =Cover crop.

Fert = Fertilizer.

For abbreviations on cover crops see Table 2.2.

Table 2.10. Soil microbial respiration (SMR), microbial biomass C&N (MBC & MBN), potentially mineralizable (PMN) and dissolved organic carbon (DOC) as affected by cover crops and mineral fertilizer on at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)									
	0–5					5-15				
	SMR	MBC	MBN	PMN	DOC	SMR	MBC	MBN	PMN	DOC
	μg C g^{-1} soil					μg C g^{-1}				
Cover crop	10 d^{-1}		μg C g^{-1} soil		mg kg^{-1}	soil 10 d^{-1}		μg C g^{-1} soil		mg kg^{-1}
CF	152	403	57 c	7.7	61	81abc	230	3.4	2.6	54
DCSB	173	441	86 a	6.2	67	76 c	273	3.7	3.0	52
SL	177	388	77abc	6.0	66	77 bc	246	1.8	3.1	59
SNL	196	422	75abc	7.5	64	88 a	268	4.8	3.5	54
WL	185	435	73bc	11.0	67	87ab	245	4.2	4.5	58
WNL	192	401	80ab	7.9	70	79 abc	260	2.2	3.3	55
LSD (P<0.1)	NS	NS		NS	NS		NS	NS	NS	NS
Fertilizer										
0 N kg ha^{-1}	177	426	76	7.4	66	82	250	3.2	3.6	55
90 N kg ha^{-1}	182	403	73	8.0	66	81	257	3.5	3.1	55
LSD (P<0.1)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	P > F					P > F				
Cover crop	0.233	0.770	0.0005	0.108	0.455	0.023	0.318	0.163	0.280	0.585
Fertilizer	0.795	0.347	0.321	0.437	0.240	0.786	0.521	0.2413	0.5334	0.746

Means in column with the same letter(s) are not significantly different at P < 0.1.   NS= Not significantly different.
For abbreviation on cover crops see Table 2.2.

Table 2.11. Soil microbial respiration (SMR), Microbial biomass C&N (MBC & MBN), potentially mineralizable (PMN) and dissolved organic carbon (DOC) as affected by the interaction of cover crops and mineral fertilizer at different depths at Ashland Bottoms, Kansas, 2014.

	Depth(cm)									
	0-5					5-15				
Interactive effect	SMR	MBC	MBN	PMN	DOC	SMR	MBC	MBN	PMN	DOC
CC x F (kg ha^{-1})	µg C g^1 soil 10 d^{-1}	…………µg C g^{-1} soil……			mg kg^1	µg C g^{-1} soil 10 d^{-1}	……µg C g^{-1} soil…….			mg kg^1
CF + 0 N	153	425	61	7.1	62	84	243	4.0 abc	2.9	56
CF +90 N	151	380	53	8.3	61	78	217	2.8 bcd	2.3	52
DCSB + 0 N	176	479	89	5.8	65	76	262	1.9 cd	3.1	53
DCSB + 90 N	171	403	81	6.5	68	75	283	5.4 ab	3.0	51
SL + 0 N	176	388	79	5.8	69	77	238	1.0 d	3.4	60
SL+90 N	178	388	74	6.2	63	78	254	2.7 bcd	2.8	57
SNL + 0 N	186	423	71	5.9	63	87	244	3.5 bc	3.4	51
SNL+90 N	206	420	79	9.1	66	89	292	6.2 a	3.5	57
WL + 0 N	191	428	79	13	68	91	252	5.7 ab	5.6	54
WL+ 90 N	180	443	66	8.5	66	83	237	2.7bcd	3.4	62
WNL + 0 N	184	415	77	9.7	69	76	284	2.8 bcd	3.1	55
WNL + 90 N	201	386	83	6.1	72	83	235	1.5 cd	3.4	54
LSD (P<0.1)	NS	NS	NS	NS	NS	NS	NS		NS	NS
	P > F					P > F				
Crop cover x fertilizer	0.324	0.894	0.384	0.114	0.528	0.521	0.235	0.029	0.571	0.402

Means in column with the same letter(s) are not significantly different at P < 0.1.

NS= Not significantly different. CC = Cover crop. Fert = Fertilizer.

For abbreviations on cover crops see Table 2.2.

Table 2.12. Gram negative and Gram positive bacteria, actinomycete, fungi and arbuscular mycorrhizae fungi (AMF) as affected by cover crops and mineral fertilizer at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)									
	0-5					5-15				
	Gram-ve	Gram+ve	Actino	Fungi	AMF	Gram-ve	Gram+ve	Actino	Fungi	AMF
Cover crop					nmol^{-1}					
CF	8.7	10.1	0.39 a	6.2	1.5	2.7	5.4 ab	0.19	1.6	0.67
DCSB	5.8	10.1	0.38 a	6.1	1.5	2.9	6.4 a	0.23	2.2	0.63
SL	6.5	9.3	0.35 ab	5.2	2.4	3.4	5.9 ab	0.23	1.9	0.63
SNL	9.7	9.4	0.32 bc	5.2	1.8	1.5	5.1 b	0.26	1.8	0.60
WL	7.8	10.9	0.32 bc	5.1	1.5	3.2	6.5 a	0.24	2.1	0.58
WNL	6.6	11.3	0.28 c	4.9	1.8	2.9	5.7 ab	0.22	1.8	0.50
LSD (P<0.1)	NS	NS		NS	NS	NS		NS	NS	NS
Fertilizer (kg ha^{-1})										
0 N	7.3	10.4	0.34	5.7	1.7	2.7	5.4	0.22	1.8	0.57
90 N	7.8	10.0	0.34	5.2	1.9	2.9	6.2	0.24	2.0	0.63
LSD (P<0.1)	NS	NS		NS	NS	NS	NS	NS	NS	NS
	P > F					P > F				
Cover crop	0.676	0.29	0.054	0.455	0.562	0.143	0.046	0.527	0.598	0.272
Fertilizer	0.765	0.428	0.967	0.240	0.486	0.414	0.337	0.669	0.909	0.261

Mean in column with the same letter (s) are not significantly different at P<0.1.
NS= Not significantly different.
For abbreviations on cover crops see Table 2.2.

Table 2.13. Gram negative and gram positive bacteria, actinomycete, fungi, and arbuscular mycorrhizae fungi (AMF) as affected by the interaction of cover crops and mineral fertilizer at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)										
	0 -5						5 -15				
Interactive effect	Gram-ve	Gram+ve	Actino	Fungi	AMF		Gram-ve	Gram+ve	Actino	Fungi	AMF
CC x Fert (kg ha^{-1})						nmol^{-1}					
CF + 0 N	6.7	10.1	0.32	7.0	1.6		2.9	7.0	0.20 bc	1.4	0.45
CF +90 N	10.7	10.1	0.31	6.5	1.6		2.5	6.8	0.20 bc	1.7	0.56
DCSB + 0 N	5.5	10.0	0.35	6.0	1.5		2.8	6.7	0.25 ab	1.9	0.6
DCSB + 90	6.0	10.3	0.4	5.7	1.5		3.1	6.3	0.21 bc	2.4	0.75
SL + 0 N	7.7	11.4	0.34	5.4	1.9		3.2	6.1	0.27 a	2.2	0.71
SL+90 N	5.4	7.2	0.3	5.4	3.0		3.7	5.9	0.19 bc	1.7	0.56
SNL + 0 N	7.5	9.4	0.28	5.2	1.8		1.9	5.9	0.24 ab	1.8	0.56
SNL+90 N	11.9	9.4	0.29	5.1	1.7		1.2	5.6	0.28 a	1.8	0.6
WL + 0 N	9.1	11.1	0.4	5.1	1.6		2.5	5.4	0.21 bc	2.1	0.63
WL+ 90 N	6.6	10.7	0.39	5.0	1.5		3.9	4.9	0.27 a	2.2	0.63
WNL + 0 N	7.3	10.6	0.34	4.7	1.7		2.7	4.6	0.18 c	1.4	0.49
WNL + 90 N	6.0	12.0	0.36	4.3	1.9		3.1	4.6	0.27 a	2.1	0.71
LSD (P<0.1)	NS	NS	NS	NS	NS		NS	NS		NS	NS
	P > F						P > F				
Cover crop x Fert	0.324	0.169	0.850	0.733	0.850		0.491	0.352	0.010	0.163	0.398

Mean in column with the same letter (s) are not significantly different at P < 0.1. NS= Not significantly different. CC = Cover crop. Fert = Fertilizer. For abbreviations on cover crops see Table 2.2
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Table 2.14. Total PLFA and Fungi:Bacteria ratio (F:B) as affected by cover crop and mineral fertilizer at different depths at Ashland Bottoms, Kansas, 2014.

| | Depth (cm) | | | |
| | 0-5 | | 5-15 | |
Treatment	Total PLFA	Fungi:Bacteria Ratio	Total PLFA	Fungi:Bacteria Ratio
Cover crop	nmol^{-1}		nmol^{-1}	
CF	34.0	0.34 b	21.2	0.13
DCSB	30.5	0.42 b	16.9	0.17
SL	29.2	0.87 a	16.0	0.16
SNL	33.1	0.40 b	16.0	0.16
WL	37.9	0.39 b	21.4	0.14
WNL	32.9	0.50 b	14.1	0.16
LSD (P<0.1)	NS		NS	NS
Fertilizer (kg ha^{-1})				
0 N	33.5	0.41	17.4	0.29
90 N	32.4	0.56	17.8	0.28
LSD (P<0.1)	NS	NS	NS	NS
		P > F		
Cover crop	0.411	0.089	0.181	0.591
Fertilizer	0.655	0.135	0.852	0.889

Mean in the same column with the same letter(s) are not significantly different at P < 0.1.

NS = Not significantly different.

CC = Cover crop.

For abbreviation on cover crop see Table 2.2.

Table 2.15. Total PLFA and Fungi:Bacteria ratio (F:B) as affected by interactive effect of cover crop and mineral fertilizer at different depths at Ashland Bottom, Kansas, 2014.

	Depth (cm)			
	0-5		5-15	
Interactive effect	Total PLFA	Fungi :Bacteria Ratio	Total PLFA	Fungi: Bacteria Ratio
CC x Fert (kg ha^{-1})	nmol^{-1}		nmol^{-1}	
CF + 0 N	30.9	0.39 b	27.2 a	0.26 abc
CF +90 N	37.1	0.30 b	14.9 bcd	0.16 c
DSB + 0 N	30.1	0.42 b	15.1 bcd	0.29 ab
DSB + 90 N	31.0	0.41 b	18.2 abc	0.31 ab
SL + 0 N	35.7	0.42 b	16.3 bcd	0.31 ab
SL+90 N	22.7	1.3 a	15.5 bcd	0.27 ab
SNL + 0 N	30.5	0.43 b	11.9 d	0.33 a
SNL+90 N	35.7	0.37 b	19.3 abc	0.26 abc
WL + 0 N	40.5	0.40 b	20.0 ab	0.27 ab
WL+ 90 N	35.3	0.37 b	22.8 ab	0.21 bc
WNL + 0 N	33.2	0.42 b	12.7 cd	0.26 abc
WNL + 90 N	32.5	0.59 b	17.1 bcd	0.29 bc
LSD (P<0.1)	NS			
	P > F		P > F	
CC x Fertilizer	0.230	0.0578	0.096	0.055

Mean in the same column with the same letter(s) are significantly different at P < 0.1.
NS= Not significantly different.
CC = Cover crop.
Fert = Fertilizer.
For abbreviations on cover crop see Table 2.2.

Chapter 3 - Impact of Direct Seeding Mulch-Based Cropping Systems on Soil Health in the Guinea Savanna Zone of Ghana

Abstract

Declining soil fertility is a major constraint to agriculture productivity in Northern Ghana. Adoption of sustainable soil intensification practices such as direct seeding mulch–based cropping systems (DMC) can potentially reverse the trend and provide food for the growing population. The objective of this study was to assess the effect of DMC on soil health. The experiment was conducted at the Savannah Agriculture Research Institute (SARI) located in Nyankpala, Ghana. The experimental design was a split plot arranged in randomized complete block design. The main treatments consisted of five different cropping systems: DMC1 (maize and *Stylosanthes guianensis*), DMC2 (maize and Black Dolichos lab lab), DMC3 (maize and cowpea), CC1 (mixed strand of *Braccharia ruziziensis, Stylosanthes guianensis, Crotalaria juncea, Crotalaria retusa*) and CK (maize as check). The sub-plot factor was fertilizer using NPK fertilizer at the following rates: 0-0-0 (control), 30-30-15 (half recommended rated) and 60-60-30 (full recommended rate) kg ha^{-1}. Soil samples were taken from 0-5 and 5-15 cm depth in June 2014. Analyses included potentially mineralizable N (PMN), soil organic carbon (SOC), Total N (TN), microbial respiration, microbial biomass, and soil pH. Biomass yield (kg ha^{-1}) was recorded at harvest. DMC cropping systems yielded significantly higher biomass compared to CK in 2012 and 2013. Soil microbial biomass and activity was not affected by the treatments. High plant biomass produced by DMC1 and DMC3 did not increase SOC and PMN relative to CK. Fertilizer application significant increased plant biomass production resulting in a significant increase in SOC and PMN in the 0-5cm depth. DMC cropping systems and fertilizer decreased soil pH in 0-5cm depth. In conclusion, both organic and inorganic soil amendments

may be utilized to improve soil chemical and biological properties in the Guinea Savanna zone of Ghana however low soil pH could hinder improvement in soil health.

Introduction

The major bio-physical constraint to agriculture productivity in Sub-Saharan Africa (SSA) is declining soil fertility (Sanchez, 2002). In West Africa, increasing human population growth has led to decrease in fallow periods, conversion of arable lands for housing and continuous cropping without sufficient inputs (Bationo et al., 2007; Adjei-Nsiah et al., 2008; Adjei-Nsiah, 2012). The results of these practices are depletion of soil nutrients and organic matter, as well as hunger and child malnutrition. Furthermore, the impacts of climate change, water scarcity, and erratic rainfall patterns have put additional stresses on existing challenges. The use of unsustainable soil management practices such as excessive tillage, slash and burn farming systems, crop residue removal, and nutrient mining (Sanginga and Woomer, 2009; Sime et al., 2015) has led to a decline in crop productivity with grain yields less than 1 Mg ha^{-1} (Montpellier report, 2014). However, the use of manure and mineral fertilizer can potentially reverse the trend of soil degradation and restore soil fertility of degraded soils in West Africa (Iwuafor et al., 2002; Vanlauwe, 2004; Bationo et al., 2007). Similarly, research in the Guinea Savana Zone of Nigeria has shown that application of manure, mineral fertilizer or combination of both have significantly impacted crop yield and soil fertility (Pieri, 1992; Vanlauwe et al., 2001a; Vanlauwe et al., 2001). The application of mineral fertilizer supplies crops with readily available nutrients. The application of manure increases soil organic matter. This affects the soil physical, chemical and biological properties. Moreover, manure provides the substrates needed for soil microorganisms to carry out their metabolic activities and nutrient cycling. The use of manure among West African farmers is limited due to low populations of livestock (Vanlauwe et al., 2001), free range of livestock management and the use of manure for fuel. Therefore an

alternative source of generating organic inputs needs to be identified and integrated into smallholder cropping systems.

Adoption of sustainable soil intensification management practices such as cover cropping and direct seed mulch-based cropping systems (DMC) can generate organic inputs needed to address the challenges of soil nutrient depletion and low crop productivity. Direct seeding mulch-based cropping system (DMC) is a conservation agriculture practice that focuses on three principles: (1) no-till or minimum-till; (2) permanent plant cover; and (3) relevant crop sequence or crop rotation (Scopel et al., 1999; Thierfelder and Wall 2009; Sime et al., 2015). The organic residue in DMC systems improves soil physical, chemical and biological properties (Scopel et al., 2005; Thierfelder and Wall 2009; Affholder et al., 2009). Direct seeding mulch-based cropping system (DMC) has the potential to increase soil organic carbon and to restore soil fertility (Baudoin et al., 2009). Additionally, DMC increases microbial biomass and enhances soil microbial activity and nutrient cycling in degraded soils (Scopel et al., 1999; Thierfelder and Wall, 2009; Baudoin et al., 2009). When legume cover crops are used in the rotation, biological nitrogen fixation (BNF) through symbiotic association with rhizobium adds approximately 60-120 kg N ha^{-1} yr^{-1}. Biological N-fixation reduces capital expenditure on chemical fertilizer (Giller, 2001; Sanginga and Woomer, 2009). The additional mulch on the soil surface reduces run off and soil erosion (Scopel, 2005; Sime et al., 2015). The mulch also protects the top soil from sealing and crusting, which increases water infiltration into the soil (Scopel et al., 1999; Thierfelder and Wall 2009; Affholder et al., 2009). Moreover, DMC enhances crop water use efficiency by conserving soil water thus enhancing climate resilience. Soil aggregation and structure is also improve because of lower soil disturbance (Affholder et al., 2009; Baudoin et

al., 2009; USDA, 2015). Thus the DMC system leads to an overall improvement in soil quality and soil health.

Healthy soil has the ability to function as a living system to sustain biological and animal productivity, maintain water and air quality, and support human health and habitation in a given natural or managed ecosystem boundaries (Arshad and Martin, 2002; Karlen et al., 2008; Lehman et al., 2015). Healthy soil functions include: (1) produce healthy plants; (2) cycle and retain nutrients such as N and C; (3) provide habitat for soil organisms; (4) serve as a reservoir for biodiversity; (5) supply plants and soil organisms with air and water for survival; (6) maintain water quality and protect water from contamination by nutrients and pathogens; (7) provide physical support for vegetation; (8) buffer against toxic accumulation and transport of natural and synthetic compounds; and (9) filter elements to protect animals, plants, and the environment from undesirable exposure (Magdoff and Weil, 2004; Lehman et al., 2015).

The concept of soil health is fairly new in northern Ghana, because maintenance of soil fertility largely focused on improvement of soil chemical properties. Further, there is scant information on how sustainable cropping systems such as DMC and cover crops affect soil health. In addition there is little or no available information on soil biology and its influence on nutrient cycling, soil organic matter and soil structure with respect to soil health. To address these challenges, this study was undertaken to determine the effect of DMC on soil health. The specific objectives were:

1. To determine the effect of DMC on soil biological and chemical properties

2. To determine the effect of nutrient management on soil biological and chemical properties.

Materials and Methods

Experimental Site

The experiment was conducted during the 2014 cropping season at the upland fields of CSIR-Savanna Agricultural Research Institute (CSIR-SARI) in Nyankpala (9°24'17.1"N; 0°57'15.1"W), Ghana. The rainfall pattern is monomodal and spreads over a period of 5-6 months (April–October) with a yearly average of 1000 mm (SARI, 2001). The yearly average temperature is 28°C. February and March are the hottest months with a daily maximum temperature of about 42°C. While December and January are the coldest months with daily minimum temperatures of about 20°C (SARI, 2001). The area experiences the impact of the cold dry North-Easterly Trade winds ('Harmattan' winds) from the Sahara Desert. The relative humidity values lies in the range of 40% to 50% (SARI, 2001).

The soil at the experimental site consists of Ferric luvisols-Gleyic plinthosols (FAO-UNESCO, 2002), which belongs to Changnayili series (A1) and Nyankpala series (A2) (Agyare, 2004). The soil is sandy silt loam and is derived from concretionary ground water laterite (Baba et al., 2013). The soil pH was 5.8 with low moisture retention. In the rainy season seepage water accumulates on top of the laterite soil.

The soil has lost its native vegetation due to annual bush fires. Currently, the vegetation that these soils support consist of farm regrowth of *Hannoa undualate* and *Daniella olivers* as the characteristic trees. Other common economic trees are *Parkia Oliveri* and *Butyrospermum parkii (*Agyare, 2004). Staples food crops grown on these soils include maize, rice, sorghum, millet, cowpea, cassava, yam, groundnut and soybean (Agyare, 2004).

Before the establishment of the trial in 2012, the field was previously used for traditional upland rice cultivation without any soil amendments and was abandoned due to low soil fertility

and soil degradation (Baba et al., 2013). Baseline soil samples were collected from 0-20 cm depth and analyzed before the trials were established in 2012. (Table 3.1).

Experimental Design

The experimental design was two-way factorial experiment in split plot, arranged in a randomized completed block design (RCBD) with three replications. Each split-block measured 90 x 3.6 m and was divided into three subplots of dimensions 30 m x 3.6 m. Each block was also separated by direct seeding mulch-based cropping systems (DMC) and with each cropping system present in the same block every year. The DMC systems adopted were strictly no-till with a three year crop rotation. The main treatment consisted of a control/check (CK) which was maize and four DMC systems. The four DMC systems consisted of:

(1) Cover crop (CC1) which comprised of each row *Braccharia ruziziensis, stylosanthes guianensis, Crotalaria juncea and Crotalaria retusa* planted in June. Pendimethaline (1500 g a. i.ha^{-1}) was sprayed after planting to control weed.

(2) DMC1 consisted of maize and stylosanthes (*Stylosanthes guianensis)* planted concurrently in June. Pendimethaline (1500 g a. i.ha^{-1}) was sprayed after planting to control weeds.

(3) DMC 2 also consisted of maize planted in June and Black *Dolichos lab lab* intercropped into the maize, 25 days after planting. Atrazine (1000 g a.i. ha^{-1}) was sprayed after planting maize to control weeds.

(4) DMC 3 comprised of maize planted in June and cowpea (short duration) intercropped into maize 25 days after planting. Atrazine (1000 g a. i. /ha) was sprayed after planting maize as pre-emergence herbicides.

The maize variety planted was "Obatampa" with a maturity period of 120 days. The maize and cowpea seed was planted using hand dibbling (manual planting using stick) while the cover crop seeds were planted by broadcasting. All cover crops in the DMC systems were not terminated during non-cropping season (November-April). The maize residues were also left on the soil to serve as mulch.

The sub-plot factor consisted of three NPK fertilizer rates broadcasted:

F0 = 0-0-0 (control)

F1 = 30-30-15 kg NPK ha^{-1} (half recommended rate) and

F2 = 60-60-30 kg NPK ha^{-1} (full recommended rate) applied using compound NPK fertilizer.

Biomass Yield

Plants biomass was randomly sampled from each plot using a quadrat 1m x1m at harvest. Fresh biomass weight was recorded, oven dried at 80°C for 72 h and then re-weighed. Biomass yield (g m^{-2}) was determined on dry matter basis and expressed as kg ha^{-1}.

Soil Sampling

Soil samples were collected in June 2014 from the DMC trial before planting of maize. Eight cores were randomly sampled using probes to a depth of 0-5 and 5-15 cm across each plot. The field moist soil samples were mixed thoroughly and air dried for three (3) days at room temperature of 25°C. The air dried soil was passed through a 2-mm mesh screen to remove stones and other debris. The processed soil samples were stored in a plastic bags at 4 C° until further analysis.

Prior to analyses about 10g of the each soil sample was weighed for gravimetric water content. The gravimetric water content was used to adjust the soil water content equivalent to 60% water filled pores (WFP) for each soil sample by re-wetting using de-ionized water.

Soil Respiration and Microbial Biomass C and N

Microbial biomass C and N was determined using the fumigation-incubation method (Jenkinson and Powlson, 1976). Duplicate 15g of air-dried soil was placed into 125-mL Erlenmeyer flasks. Soils were re-wetted to 60% WFP using de-ionized water. The samples were then pre-incubated for seven days at 25^0C. After the pre–incubation period, germinated plant roots or shoots were removed from the soils using forceps. The moisture content was adjusted to 60% WFP. One set of samples was fumigated with chloroform in a vacuum desiccator containing a wet paper towel and a beaker with 70-mL of ethanol-free chloroform and boiling chip. The desiccator was sealed and evacuated three times for approximately 2 min per session to allow the chloroform to vaporize. After the third evacuation, the desiccator was tightly closed (sealed) to allow the chloroform to diffuse into the soil. Both the fumigated and the non-fumigated samples were kept in a dark room at 25^0C. After 22 h, the beaker and paper towel were removed and the desiccator was evacuated ten times for 3 min each time. Both flasks samples were then placed in 940-mL mason jars containing enough (50-mL) water to maintain a moist environment. The mason jars were tightly closed and incubated for 10 days at 25°C, after which the headspace CO_2-C concentration was measured using a gas chromatograph (Shimadzu GC-8A, Shimadzu Scientific Instruments, Columbia, MD).

After the 10-d incubation, 60 mL of 1 M KC1 was added to each sample, and shaken for 1 hr on a digital shaker at 300 rpm. The suspensions were filtered through Whatman filter paper No. 42 (110 mm) into 20 mL scintillation vials, and stored in a freezer until analyzed for

inorganic N (NH_4-N and NO_3-N) using colorimetric procedure (Maynard et al., 2006) in the Kansas State Soil Testing Laboratory.

Soil microbial respiration (SMR) was measured as the cumulative evolved C-CO_2 after the 10-d incubation from the non- fumigated samples using gas chromatography as describe earlier (Gajda and Martyniuk, 2005).

Microbial biomass carbon (MBC) was calculated as the difference between CO_2 evolved from fumigated and non-fumigated soils after the 10 day incubation period and divided by a conversion factor of K_C =0.45 (fraction of biomass C mineralized into to CO_2) (Jenkinson and Powlson, 1976; Gajda and Martyniuk, 2005).

Microbial biomass nitrogen (MBN) was calculated as the difference between NH_4-N and NO_3-N evolved from fumigated and non-fumigated soil samples after the 10-d incubation period, and divided by a conversion factor of $K_N = 0.54$ (Jenkinson and Powlson, 1976).

Potentially mineralizable nitrogen (PMN) was determined as the difference between initial soil NH_4-N and NO_3-N and the NH_4-N and NO_3-N from the incubated non- fumigated (control) samples (Gugino et al., 2009).

Chemical Analysis

Soil pH was determined using 1:1 soil–water suspension. Soil available P was measured using Mehlich 3 extraction (Frank et al., 1998). Soil exchangeable cations K^+, Mg^{2+}, and Ca^{2+} were extracted with ammonium acetate. Soil Mg^{2+} and Ca^{2+} were analyzed using atomic absorption /emission spectrometry while K^+ was analyzed using flame photometry (Warncke and Brown, 1998). Soil inorganic N was extracted by adding 100 mL of 1M KCl to 25g of field moist soil. The samples were then shaken for 60 min on orbital shaker at 300 rpm, and

filtered through Whatman No. 42 filter paper. The extractant was analyzed for NH_4-N and NO_3-N using a colorimetric analysis (Maynard et al., 2006).

Soil Organic Carbon (SOC) and Soil Total Nitrogen (TN)

Air-dried soil samples were ground to a fine powder using a mortar and pestle after removing roots. The ground soil samples were sieved through a 53 µm screen. The soil samples were analyzed for soil organic C and total N content by dry combustion using a C/N Analyzer (EA 112) (Mikha and Rice, 2004).

Data Analysis

Data analysis was done using Proc mixed model in SAS 9.4 (SAS institute, Inc. 2014). Unless otherwise stated, treatments means were separated using Fisher protected least significant difference (LSD) at a significance level of $P<0.05$.

Results

Soil Health Biological Indicator

Three years after the establishment of the trial, assessment was made on selected biological indicators of soil health; SMR, MBC, MBN, PMN, SOC, TN as well as biomass yield to determine how they influence soil health. Apart from biomass yield that was taken on the field, all the other parameters examined in this study were done in the laboratory.

Biomass yield (kg ha⁻¹)

Biomass yield was significantly (P<0.0001) affected by the interactive effect of cropping systems and fertilizer in 2012 but not in 2013. In 2012 each of the cropping systems showed different responses to the mineral fertilizer applied. Biomass yield under DMC2 showed linear respond to mineral fertilizer increment (Fig. 3.3) Similarly, biomass yield for CC1 and DMC3 showed greater response when 30-30-15 kg NPK ha⁻¹ was applied and then remained fairly linear when the 60-60-30 kg NPK ha⁻¹ was applied. The check (CK) initially showed a slow response to when 30-30-15 kg NPK ha⁻¹ was applied but thereafter recorded a greater increase (exponential increase) in biomass yield when 60-60-30 kg NPK ha⁻¹ was applied (Fig 3.3). In 2013, biomass yield under all the cropping systems showed a nearly linear respond to mineral fertilizer although not significant (Fig. 3.4). Greater responses were observed when the full recommended fertilizer rate (60-60-30 kg NPK ha⁻¹) was applied. In general there was a strong relationship between the amounts of plant biomass produced by each of the cropping system and mineral fertilizer applied (Fig 3.3 and Fig. 3.4).

Plant biomass yield was also significantly affected by cropping systems in both years (2012 & 2013). In 2012, DMC1 and DMC3 produced significantly greater (P<0.001) biomass relative to CK (Fig. 3.1). In 2013, similar trends were observed except all DMC cropping

systems yielded significantly (P<0.001) more biomass than the CK cropping system (Fig. 3.1). Thus DMC systems were capable of generating more organic inputs needed to drive nutrient cycling in an ecosystem. Similarly, the effect of mineral fertilizer on biomass production was significantly (P< 0.001) for both years (2012 and 2013) (Fig. 3.2). In general an increase in mineral fertilizer resulted in a corresponding increase in plant biomass production. The full recommended fertilizer rate (60-60-30 kg NPK ha^{-1}) produced the greatest biomass relative to the control (0-0-0 kg NPK ha-^{1}) (Fig. 3.2). Plant biomass production was driven by the amount of fertilizer applied rather than precipitation received per cropping season (Fig. B.1). Thus adequate fertilizer is need to produce high levels of biomass.

Soil organic carbon (SOC)

At the 0-5 cm depth, soil organic carbon (SOC) was significantly (P<0.10) greater in the CK cropping system than the DMC cropping systems (Fig. 3.5). Whereas, at the 5-15 cm depth, there was no significant (P<0.10) difference in SOC among cropping systems (Fig. 3.5). The CC1 cropping system has the highest SOC (~6.5 g C kg^{-1}) compared to the other cropping systems in the 5-15 cm depth. Soil OC was significantly (P<0.10) increased by fertilizer application at both depths. The full recommended rate of fertilizer (60-60-30 kg NPK ha^{-1}) resulted in greater increased in SOC compared to the control (0-0-0 kg NPK ha^{-1}) (Fig. 3.6). The incremental increase in SOC due to fertilizer applied with respect to the control was about 21% in the 0-5 cm and 13% in the 5-15 cm depths respectively. The interactive effect of fertilizer and cropping systems was not significantly (P<0.10) different for SOC irrespective of depth. However, CK + 60-60-30 kg NPK ha^{-1} recorded the highest SOC of 8.1 g C kg^{-1} in the 0-5 cm depth. This may be due to the least soil pH decline under CK cropping system. Generally, the trend also shows that SOC increases with increasing fertilizer rates at 0-5 cm depth. (Fig. 3.7).

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This may be due to presence of organic residue on soil which contributed to higher SOC. In general SOC decreased with increasing fertilizer rates at 5-15 cm (Fig. 3.8).

Soil Total Nitrogen (TN)

Soil TN was not significantly (P<0.05) affected by cropping systems irrespective of depth. The CK and CC1 cropping systems had high TN compared to the other cropping systems in 0-5 cm and 5-15 cm depths, respectively (Fig. 3.9). At the 0-5 cm depth, TN was not significantly (P<0.05) affected by fertilizer (Fig. 3.10). In contrast, at the 5-15 cm depth, TN had significantly (P<0.05) higher levels at the 60-60-30 kg NPK ha^{-1} (full recommended fertilizer rate) which was 21% greater relative to the 0-0-0 kg NPK ha^{-1} (control). It is likely that the N assimilated by the plant was returned as residual plant N. The interactive effect of cropping system and fertilizer application was not significant for TN in both 0-5 and 5-15 cm depths (Table 3. 4).

Soil Microbial Respiration

Soil microbial respiration was not significantly (P<0.05) affected by the cropping systems irrespective of depth (Table 3.2). In generally, higher soil microbial respiration was observed in the 0-5 cm compared to the 5-15 cm depth. Soil microbial respiration was significantly (P<0.05) greater at 30-30-15 kg NPK ha^{-1} (full recommended rates) compared to 0-0-0 kg NPK ha^{-1} (half recommended rates) in the 0-5 cm depth. Microbial respiration was not significantly (P<0.05) different in the 5-15 cm depth. The interactive effect of fertilizer and cropping systems was not significant (P<0.05) irrespective of depth (Table 3.3). Generally, higher soil respiration was observed in the 0-5 cm compared to 5-15 cm depth. This could be due to availability of nutrients from plant residues and higher soil microbial population in the 0-5 cm surface layer.

Microbial Biomass C

Microbial biomass carbon (MBC) was not significantly (P<0.05) affected by cropping systems or fertilizer application (Table 3.2). At the 0-5 cm depth, MBC was 44% and 38% greater in the CK cropping system relative to DMC1 and CC1 cropping systems, respectively. At 5-15 cm depth, MBC was 36% higher in CC1 compared to DMC3 (Table 3.2). The effect of fertilizer application was not significant (Table 3.2). The interactive effect of cropping systems and mineral fertilizer did not significantly (P<0.05) affect MBC at the different depths (Table 3.3). Microbial biomass C ranged from 258 μg C g^{-1} soil (CK + 30-30-15 kg NPK ha^{-1}) to 139 μg C g^{-1} soil (DMC3 + 0-0-0 kg NPK ha^{-1}) in the 0-5 cm depth. In the 5-15 cm depth, MBC ranged from 153 μg C g^{-1} soil (CC1 + 60-60-30 kg NPK ha^{-1} and CC1 + 30-30-15 kg NPK ha^{-1}) to 93 μg C g^{-1} soil (DMC3 + 0-0-0 kg NPK ha^{-1}) (Table 3.3).

Microbial Biomass Nitrogen (MBN)

Microbial biomass nitrogen (MBN) was significantly (P<0.10) increased by the interactive effect of cropping systems and fertilizer in the 5-15 cm depth but was not significantly (P<0.05) different with respect to cropping systems at the different depths (Table 3.2 and 3.4). The most significant MBN of 29 and 25 μg C g^{-1} soil were found in DMC2 + 60-60-30 kg NPK ha^{-1} and DMC3 + 0-0-0 kg NPK ha^{-1} respectively compared to 13 μg C g^{-1} soil from CK + 0-0-0 kg NPK ha^{-1} which gave the least MBN. The difference could be due to effect of fertilizer applied and/or cropping systems. MBN was significantly (P<0.05) greater when 60-60-30 kg ha^{-1} and 30-30-15 kg ha^{-1} of NPK fertilizer were applied compared to the control (0-0-0 kg ha^{-1}) in the 0-5 cm depth (Table 3.2). Generally, higher MBN was recorded at the 5-15 cm depth compared to 0-5 cm depth.

Potentially Mineralizable N (PMN)

At 0-5 cm depth, potentially mineralizable N (PMN) was significantly (P<0.05) affected by cropping systems but not at 5-15 cm. Higher PMN was observed in the CK cropping system compared to the DMC1 cropping system (Fig. 3.11). This infers that CK cropping system contributed more available N. Similarly, PMN showed significant (P<0.05) variation at 0-5 cm depth with respect to fertilizer application as 30-30-15 kg NPK ha^{-1} contributed 24% more PMN compared to 60-60-15 kg NPK ha^{-1} (Fig. 3.12). On the contrary, there was no significant effect of fertilizer application on PMN at the 5-15 cm depth. Irrespective of depth, PMN was not significantly (P<0.05) affected by the interactive effect of cropping systems and mineral fertilizer application (Table 3.6). Although DMC3 + 30-30-15 kg NPK ha^{-1} and DMC3 + 60-60-30 kg NPK ha^{-1} recorded the highest mean of 15 μg C g^{-1} soil and 12 μg C g^{-1} soil for PMN at 0-5 cm and 5-15 cm depth respectively. The trend seems to suggest that PMN could be influenced by the interactive effect of cropping systems and fertilizer application (Table 3.4).

Soil Health Chemical Indicators

After three years of trial establishment, some changes were observed in the physiochemical property of the soil as result of management (Table 3.1). The key soil health chemical indicators examined in this experiment were soil pH, residual N, P, and K, and Ca^{2+} and Mg^{2+}.

Soil pH

Though the soil at the trial site had low pH (Table 3.1) at the time of the trial establishment, four years after trial establishment, significant (P<0.05) changes in soil pH were observed. At the 0-5 cm depth, DMC1 cropping system recorded the greatest (P<0.05) soil pH decline compared to CC1 and CK cropping systems (Fig. 3.13). However, at 5-15 cm, there was

no significant variation (P<0.05) in soil pH across the various cropping systems, although the trend was the same as those observed in the 0-5 cm depth. Soil pH declined with depth. Similarly, at the 0-5 cm depth, soil pH significantly decline with increasing fertilizer rates. The application of 60-60-30 kg NPK ha^{-1} (full recommended rate) reduced soil pH relative to-30-30-15 kg NPK ha^{-1} (half recommended rate) and 0-0-0 kg NPK ha^{-1}(control) plots (Fig. 3.14). Whereas at 5-15 cm depth, there was no significant difference in soil pH due to fertilizer but the trends observed were similar to those in the 0-5 cm depth (Fig. 3.14). The results show that as fertilizer rate increases soil pH decreases. This can potentially affect plant availability of nutrients as well as soil microbial activity. Finally, irrespective of depths, there were no significant changes in soil pH due to interactive effect of cropping systems and mineral fertilizer (Table 3.6).

Soil Inorganic Nitrogen, Phosphorus and Potassium (NPK)

Residual inorganic N (NH$_4^+$+NO$_3^-$) and P were not significantly affected by cropping systems irrespective depths (Table 3.5). At 0-5 cm, the DMC1 cropping system had the highest residual inorganic N (26 mg N kg^{-1}) while DMC2 recorded the lowest residual N (21 mg N kg^{-1}). Similarly, at 0-5 cm depth, mean residual P ranged from 7.3 mg kg^{-1} in CC1 cropping system to 5.2 mg kg^{-1} in DMC1. At 5-15 cm depth, DMC1 had the highest residual N of 17 mg kg^{-1} than the other cropping systems while CC1 cropping system recorded the highest residual P of 4.6 mg kg^{-1}. In general, higher concentrations of soil residual N and P were found in the 0-5 cm than 5-15 cm depths. Soil K was significantly (P<0.05) affected by the cropping systems at 0-5 cm depth. (Table 3.5). The CK cropping system had higher levels of soil residual K relative to DMC1 and CC1 cropping system. In contrast, at the 5-15 cm depth, there was no significant difference in soil K (Table 3.5).

Application of fertilizer had a significant ($P<0.05$) effect on soil inorganic N and P but not K at the 0-5 cm depth (Table 3.5). At 0-5 cm, 30-30-15 kg NPK ha^{-1} contributed more available P relative to the control. Whereas, 60-60-30 kg NPK ha^{-1} also contributed more soil residual inorganic N compared to the control (Table 3.5). At the 5-15 cm depth, soil residual N was significantly affected ($P<0.05$) by fertilizer application. The application of 60-60-30 kg NPK ha^{-1} contributed more residual N relative to the control. In general, there was more residual N at 0-5 cm than 5-15 cm. This can be due to the carry-over / residual effect of the mineral fertilizer applied (Table 3.5). At 5-15 cm, 30-30-15 kg NPK ha^{-1} fertilizer has the highest residual P however, not statistically significant (Table 3.5). Though soil K was not significantly affected by fertilizer application at 0-5 cm depth, mean values ranged from 100 mg kg^{-1} (60-60-30 kg NPK ha^{-1}) to 91 mg kg^{-1} (0-0-0 kg NPK ha^{-1}) (Table 3.5). At 5-15 cm depth, 60-60-30 kg NPK ha^{-1} fertilizer has significantly higher soil K compared to the 0-0-0 kg NPK ha^{-1}. The interactive effect of cropping systems and mineral fertilizer was not significant ($P<0.05$) for soil inorganic N, P, and K at the different depths (Table 3.6). Soil Ca^{2+} and Mg^{2+} availability were not significantly ($P<0.05$) affected by both cropping systems and the amount of fertilizer (Table 3.7). The interactive effect of cropping systems and mineral fertilizer was not significant for available Ca^{2+} and Mg^{2+} (Table 3.8).

Discussion

Effect of Soil Health Biological Indicator

Crop residue removal at harvest is the underlying cause of low agricultural productivity in most West Africa countries because effective soil management practices depend on the maintenance of soil organic matter. The quantity of organic matter in the soil is dependent upon the quantity and quality of organic inputs generated and incorporated into the soil (Dick and Gregorich, 2004). Therefore organic inputs provide the driving force for increased soil microbial activity and soil organic carbon (SOC).

Biomass yield

The interactive effect of cropping systems and mineral fertilizer and resulted in a significant increase in biomass yield in 2012. In 2013, the interactive effect cropping systems and mineral fertilizer was not significant although the trend was similar as in 2012. Averaged across years, greater increased in plant biomass due to the interactive effect of cropping systems and mineral fertilizer was higher by ~1.7 times (71%) in 30-30-15 kg NPK ha^{-1} and 2.4 (138%) times in 60-60-30 kg NPK ha^{-1} compared to where no mineral fertilizer was applied respectively. In general greater biomass yields due to the interactive response of cropping systems and mineral fertilizer was achieved when the full recommended rates (60-60-30 kg NPK ha^{-1}) was applied. The DMC1 and DMC3 yielded the greatest biomass response due to interactive effect of mineral fertilizer in 2012 and 2013, respectively. The observed trend therefore suggests that biomass was dependent on mineral fertilizer rather than the DMC cropping systems. Our results confirm the findings that on degraded soils of West Africa, regardless of the cropping systems and the production goals, mineral fertilizer application is necessary for achieving higher crop productivity (FAO, 1999; Vanlauwe et al., 2001; Giller, 2002; Bationo et al., 2007; Osundare,

2008). The mineral fertilizer supplied the growing plants with readily available nutrients which resulted in good plant establishment thereby culminating in higher biomass yield (Osundare, 2008; Chianu et al., 2012). As such it was not surprising that application of mineral fertilizer alone resulted in a significant increase in biomass yield. Averaged across years, the application of 30-30-15 kg NPK ha^{-1} and 60-60-30 kg NPK ha^{-1} increased biomass approximately 79% and 149% respectively, compared to the control. This findings affirm results from a long term study conducted in the Sudanian zone of West Africa where it was observed application of mineral fertilizers was an effective technique for increasing crop yields (Bationo et al 2007).

With regards to the cropping systems alone, DMC cropping systems produced significantly more biomass relative to the CK cropping system in both years. In 2012, the average biomass yield increment from DMC systems was on average of 84% compared to CK cropping system. Similarly, in 2013, the DMC cropping systems increased biomass by an average of 85% compared to the CK cropping system. Thus it was evident that the DMC cropping systems were more efficient in higher plant biomass production. Apart from the mineral fertilizer effect on biomass, the increment in biomass yield in both years could be attributed to the tropical legume crops integrated into the DMC cropping systems which made more N available for plant growth through BNF and perhaps due to the mineralization of the organic residue retained on soil which may release nutrients (N, P, K, S, etc) for plant growth. Moreover, presence of the organic residue probably provided a conducive environment for several mechanisms of improved agronomic efficiency. These mechanisms include improvement in soil water retention, increased soil biodiversity, and better synchronization of nutrient supply with crop demand (Ayoola and Adeniyan, 2006; Alley and Vanlauwe, 2009). Giller (2001) and Bationo et al. (2014) also reported that rotation of cereals and legumes is a cost effective means

of improving soil fertility and productivity. Thus rotation of cereals with legumes increases N use efficiency (Sanginga and Woomer, 2009; Bationo et al., 2014). Field experiments conducted at several sites in West Africa have shown cereal yield increases in cereal/ legume rotations of between 15 and 79% compared with continuous cereal systems (Bationo et al., 2014). Since sustainable agricultural management depends on maintaining soil health efficient recycling of organic material in combination with rotations of N_2-fixing legume and chemical fertilizers would be required to maintain soil fertility in the West Africa (Bationo et al. 2007; Osundare, 2008; Sanginga and Woomer, 2009). Thus it would be prudent to encourage adoption DMC cropping systems with a combination of mineral fertilizer because of it long term benefits.

Although mineral fertilizer increased biomass production, its continual usage could have detrimental effects on soil health in the long term since mineral fertilizers (N−based fertilizers) are known to have negative effect on soil pH (Rengel, 2004; Rengel, 2011). Low soil pH resulting from mineral fertilizer application is known to negatively affect microbial activity and rate of N mineralization of soil organic matter and as well the availability of plant nutrients (Narteh and Sahrawat 1997; Sanginga and Woomer, 2009). As a result of low pH, high levels of Al toxicity and P deficiency are known to occur in tropical soils particularly, those in Africa (Mokwunye et al., 1987; Sanginga and Woomer, 2009). Inclusion of the legumes in the DMC cropping system could be the reason for the soil pH decline compared to CK due to N inputs from N fixation. Peoples et al. (2004) also observed that legumes can to lead to a decline in soil pH due to nitrification of biological fixed N. It is also evident that liming would be necessary to correct low soil pH.

Soil Organic Carbon (SOC) and Total Nitrogen (TN)

Soil organic carbon was not dependent on the quality nor quantity of plant biomass. High biomass produced by the DMC (CC1, DMC1, DMC2 and DMC3) cropping systems did not result in greater SOC. These results are contrary to those reported by several authors that the quality and quantity of organic input determine to a large extent SOC (Vanauwe et al., 2001; Lal 2004; Allen et al., 2011; Deb et al., 2015). The lack of change in SOC may be due to the short time of the experiment and soil pH. Bationo et al. (2007) reported that 2 ton ha^{-1} and 4 tons ha^{-1} of crop residues used as mulch on soil increased SOC by 1.7 g kg^{-1} and 3.3 g kg^{-1} at the 0.1 m (10 cm) depth compared to unmulched soil in a study conducted in Niger, West Africa. Therefore taking 4 tons ha^{-1} of residues into consideration, all the cropping systems did not produced sufficient residues to build the soil organic carbon. Moreover, since termites are the most dominant insect found in Northern Ghana due to relatively dry weather, termites may have consumed the organic residues used as mulch (Sanginga and Woomer, 2009).

On average SOC increased ~24 % in the top soil layer (0-5 cm) under the CK cropping system compared to the DMC cropping systems. The outcomes from this study had also demonstrated that mineral fertilizer is needed to increase SOC as mineral fertilizer application resulted in significant increase in SOC at both depths. Mineral fertilizer application has been shown to increase SOC (Havlin et al., 1990; Paustian et al., 1997; Giller, 2002; Dick and Gregorich; 2004). On the contrary, Bationo et al. (2007) observed that application of mineral fertilizer decreased SOC. This may be due to improved plant residue C: N ratio of which would result in increased decomposition of the residues. Thus quantity and quality of the biomass must be considered when determining the effects of mineral fertilizer on SOC.

An experiment conducted in Northern Guinea Savanna of Burkina Faso showed that combination of organic residue and mineral fertilizer resulted in higher SOC under no-tillage (Bostick et al., 2007; Ouedraogo et al., 2007). The lack of cropping systems effect on SOC may be due to the short time frame of the experiment. Soil organic C plays a key role in soil aggregation and structure development as well as serves as a reservoir for plant nutrients, which affect soil health as well as crop productivity. The combination of mineral fertilizer and effective residues management would be a sustainable mechanism to increase SOC. This would help in restoring soil fertility and soil quality on degraded soils of West Africa (Bationo et al., 2007). We may interpret total N respond to mineral fertilizer at 5-15cm depth to be due to the effect of soil nitrate leaching.

Soil Microbial Biomass and Activity

Three years after initiating of this experiment, SMR, MBC, and MBN were not significantly affected by the cropping systems. However, these parameter (SMR, MBC) showed trends similar to SOC. Perhaps 3 yrs was too short of a period to detect significant changes in these microbial parameters on inherently degraded soils. Several authors have reported significant responses in some of these parameters in trials established more than 5 yrs under different cropping systems (Balota et al., 2003, Balota et al., 2004; Liebig et al., 2004; Balota et al., 2011; Abdollahi and Munkholm, 2014). Soil microbial respiration values were comparable to Gajda et al. (2013). Higher soil microbial respiration suggests increased microbial activity and mineralization of labile C (Parkin et al., 1996; Allen et al., 2011) which are linked to availability of substrate for the microbial community. Pertaining to soil health, high soil microbial respiration can be used as an index for good soil quality while low soil microbial respiration is an index for poor soil quality (Balota et al., 2004). However, this is dependent on favorable

environmental conditions such as moisture, temperature, soil pH, substrate availability, and nutrient concentration (Anderson and Domsch, 1993; Schloter et al., 2003; Singh et al., 2011). A significant positive response of soil microbial respiration due to mineral fertilizer applied indicates mineral fertilization improves the C: N ratio of the plant. Mean MBC value recorded in this experiment were generally low compared to those reported by other authors (Liebig et al., 2004; Gajda and Martyniuk, 2005; Balota et al., 2011; Abdollahi and Munkholm, 2014). This might be due to short time frame of the experiment and low rainfall in 2014 (Fig. B.1). High MBC found under CK and CC1 cropping systems reflects increased soil microbial activity. We interpret high MBC as greater accumulation of labile–C in the organic pool of the soil (Gajda and Martyniuk, 2005; Singh et al. 2007; Balota et al. 2011). Therefore the increased MBC found in CK and CC1 cropping systems can be taken as early signal of changes in SOC pool as reported by Abdollahi and Munkholm (2014). Similarly, we also assert the increased MBN observed in DMC2 and DMC3 to be an early indicator of change in TN. It is therefore not surprising that both MBN and TN was not significantly affected by cropping systems. The increase in MBN due to mineral fertilizer suggests that mineral fertilizer is needed to increase N mineralization from the soil organic pool.

Potentially mineralizable nitrogen (PMN)

Potentially mineralizable N was 62% greater under CK cropping system compared to DMC1 cropping systems. This demonstrates greater N mineralization potential for CK cropping systems (Liebig et al., 2004). Greater PMN values have been associated with changes in soil organic matter quality which is based on the quality of organic input introduced in the soil (Khorsandi and Nourbakhsh, 2008). Results from this study indicate the contrary, biomass or organic residue from CK cropping systems were mostly carbon dominated in terms of biomass

quality. Perhaps the increased PMN observed under CK cropping systems was influenced by SOC and soil pH as reported by Narteh and Sahrawat (1997). The increased PMN due to mineral fertilizer suggests mineral fertilizer is necessary to enhance N mineralization. Mijango et al. (2006) found higher PMN with the application of mineral fertilizer in tillage systems. The mineral fertilizer may have narrowed the C:N ratio of the organic residue resulting in increased microbial activity and N mineralization of the plant matter on the soil. In agreement with our findings, Hirzel et al. (2012) and Villasenor et al. (2015) reported that mineral fertilizer (especially N based) stimulates soil microorganisms to increase biomass development and mineralization of soil OM to release nutrients for plant uptake and utilization. In line with this, Deenik (2006) and Gajda and Przewoka (2012) also stated that soils with high PMN tend to be naturally fertile and require less agricultural inputs. However, with biomass yield, the CK cropping systems yielded the least compared to the other cropping systems. It is evident from this study that, if the PMN under cropping system is properly harnessed, then the quantity of mineral fertilizer required for good crop establishment could be reduced. This may also reduce the cost of production associated with purchasing mineral fertilizer since soils in West Africa are generally poor in nutrient especially N due to degradation (Iwuafor et al., 2002; Vanlauwe, 2004).

Soil Health Chemical Indicators

The soil health chemical indictors assessed were pH, residual inorganic N, P, K, Ca and Mg. Karlen et al. (2008) reported N, P, K, and pH as the most notable chemical indictors for assessing soil health. Since the establishment of the trial 3 yrs ago, an increase was observed in the chemical properties assessed except soil pH that decreased (Table 3.1). From our results soil inorganic N, P, K, Mg, and pH was vertically stratified under the cropping systems. The higher concentrations of inorganic N, P, K, and pH in the 0-5 cm of soil can be attributed to the effect of no-tillage and crop residue cover. Abdollahi and Munkholm (2014) observed increased concentrations of K, P, and pH in the 0-10 cm depth in a reduced tillage cover crop system. Similarly higher concentrations of K were observed in 0-13 cm depth in a reduced tillage system (Comia et al., 1994). The increase concentration of available K under the CK compared to CC1 may due to the type of crop residue used as mulch. Buerkert et al. (2001) found significant residual K on soil mulched with crop residues from millet in a study conducted in West Africa. We interpret the significant increase in soil inorganic N due to mineral fertilizer as an indication for low soil fertility with regards to N. Therefore mineral fertilizer especially N based is necessary to enhance higher crop productivity (Bationo et al., 2007) and as well as to enhanced the decomposition the organic residue (Ouedraogo et al., 2007). The significant increase in soil inorganic N and K in the subsoil due to (60-60-30 kg N ha^{-1}) mineral fertilizer may be the result of leaching due to their mobility in soil (Halvin et al., 2013). The non-significant result of soil P in the 5-15 cm (subsoil) in this study is consistent with Abdollahi and Munkholm (2014), who also observed the same trend in a cover crop study. The low P levels observed in this study are comparable to those by reported Buerkert et al. (2001). Perhaps the low pH affected P availability. Mokwunye et al. (1986) and Bationo et al. (2007) observed that in tropical soils,

low pH increased the availability of Fe^{+3} or Al^{+3} oxides which led to P fixation or deficiency. Therefore liming would be necessary to increase pH and ensure availability. Although the effect of soil pH had already been discussed, its continual decline due to mineral fertilizer application was expected. Mineral fertilizers especially N based are known to decline soil pH with time (Geisseler and Scow, 2014). An experiment conducted in West Africa revealed that continuous cultivation using mineral fertilizers decreased base saturation, increased nutrient leaching, intensified soil acidity, and increased exchangeable aluminum which decreased crop yield (Bationo et al., 2007). In conclusion Bationo et al. (2007) emphasized that use of organic inputs (green manure, cover crop, etc) would be needed to counteract the negative impact of mineral fertilizers. Therefore the effect of soil pH on crop productivity, soil health should not be underestimated.

Soil Ca^{2+} and Mg^{2+} did not show any significant responses to cropping systems as well as mineral fertilizer. Mineral fertilizer and lime would be necessary to improve the soil chemical indicators.

Conclusion

DMC1 and DMC3 cropping systems produced significantly higher plant biomass compared to the CK cropping system in 2012. In 2013, DMC cropping systems produced significantly more plant biomass compared to CK cropping system. In both years, mineral fertilizer application resulted in a significant increase in plant biomass. Plant biomass was dependent on the quantity of mineral fertilizer applied. Increased plant biomass did not increased SOC and PMN. The CK cropping system had greater increased in SOC compared to the DMC cropping systems may be due to the least decline in pH. Soil pH decline was due to mineral fertilizer and perhaps the legume N included in the DMC cropping systems. Cropping systems had no significant effect on SMR, MBC and TN and P. Meanwhile mineral fertilizer affected SMR, MBN, SOC and TN, PMN, N, P and K. Since soils in Northern Ghana are mostly degraded with low nutrients and low SOM (<1%). Combining organic residues with judicious nutrient management would be sustainable way to improve soil health in the Guinea savanna zone of Northern Ghana. As such DMC cropping systems would be the most appropriate systems since they produce greater plant biomass which serve as the basis for sustainable soil health improvement. However, low soil pH should be addressed by liming to boost higher crop productivity and soil health. Finally we recommend that the study should be repeated to validate findings, while future research could focused on the identification of the microbial community structure and soil enzymes

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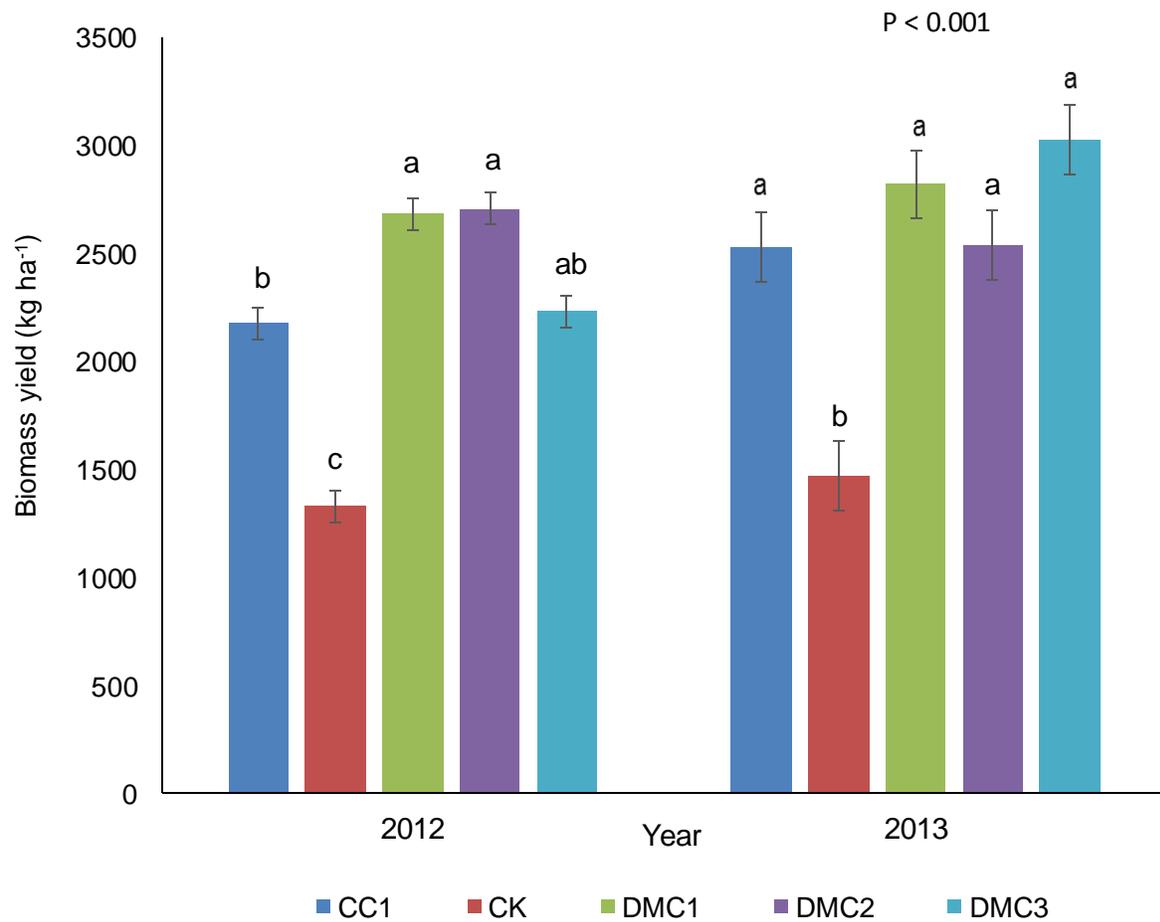
Figure 3.1. Plant biomass yield as affected by cropping systems in the year 2012 and 2013 at Nyankpala, Ghana.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
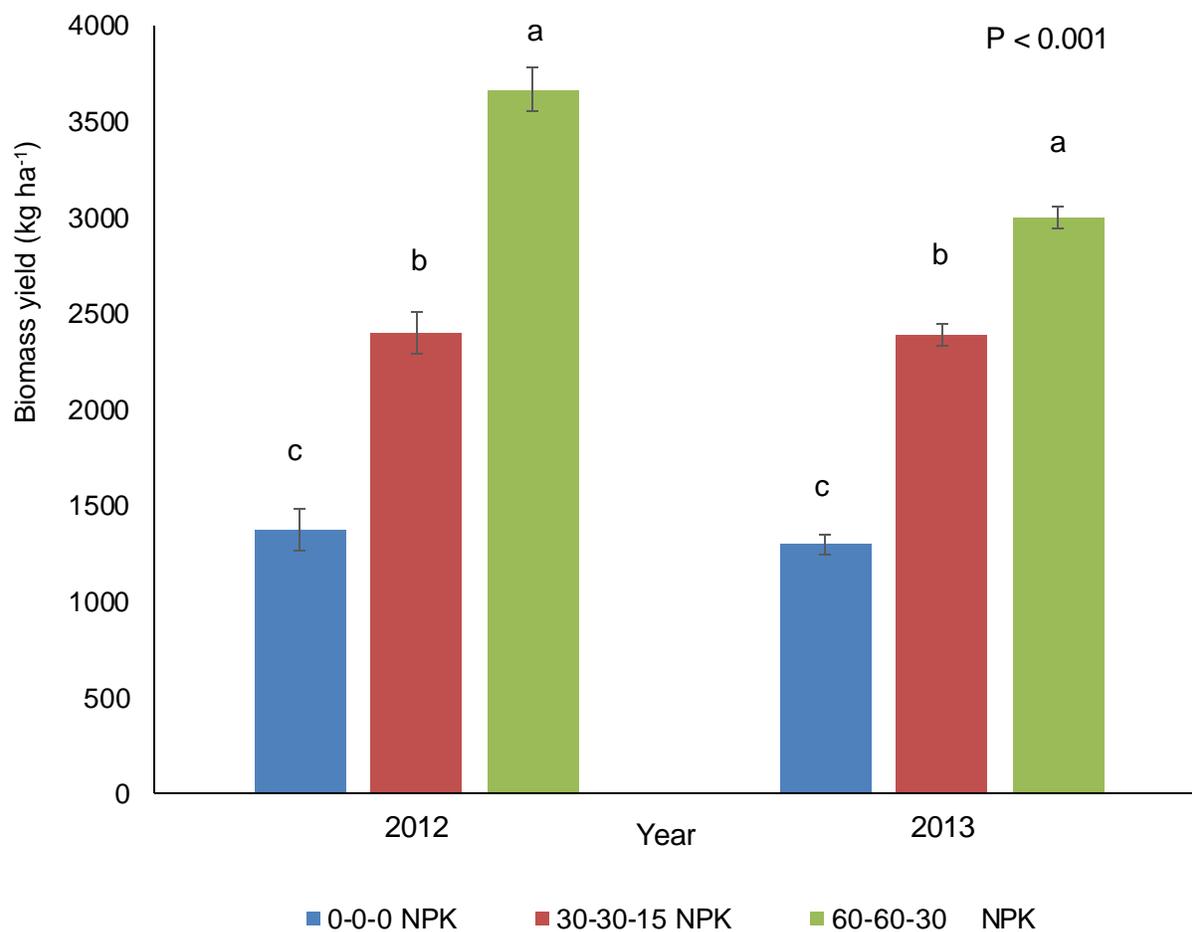DMC3 = Maize + Cowpea

Figure 3.2. Plant biomass yield as affected by of fertilizer in the year 2012 and 2013 at Nyankpala, Ghana.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
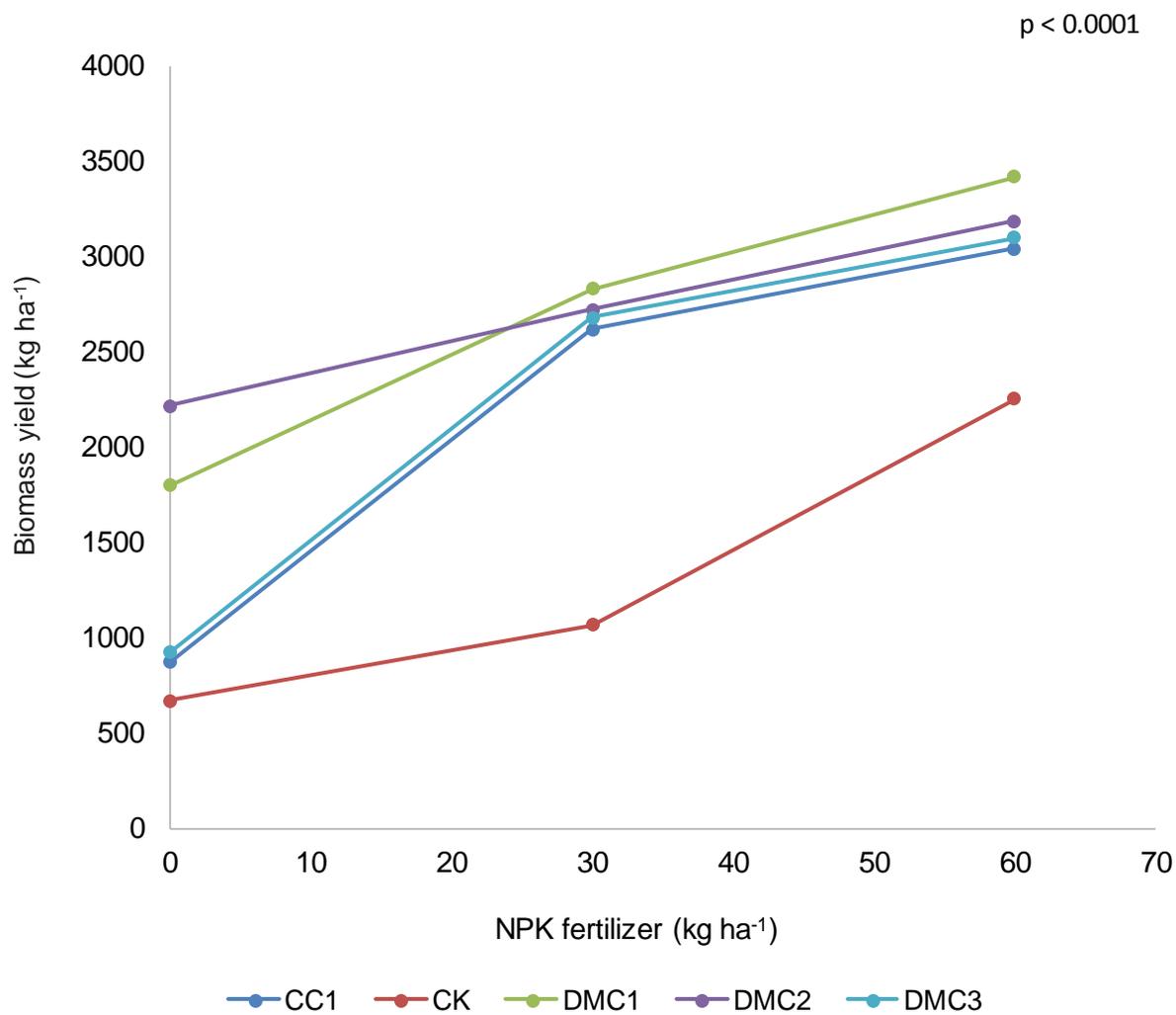DMC3 = Maize + Cowpea

Figure 3.3. Plant biomass yield as affected by the interactive effect of cropping systems and fertilizer in 2012 at Nyankpala, Ghana.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
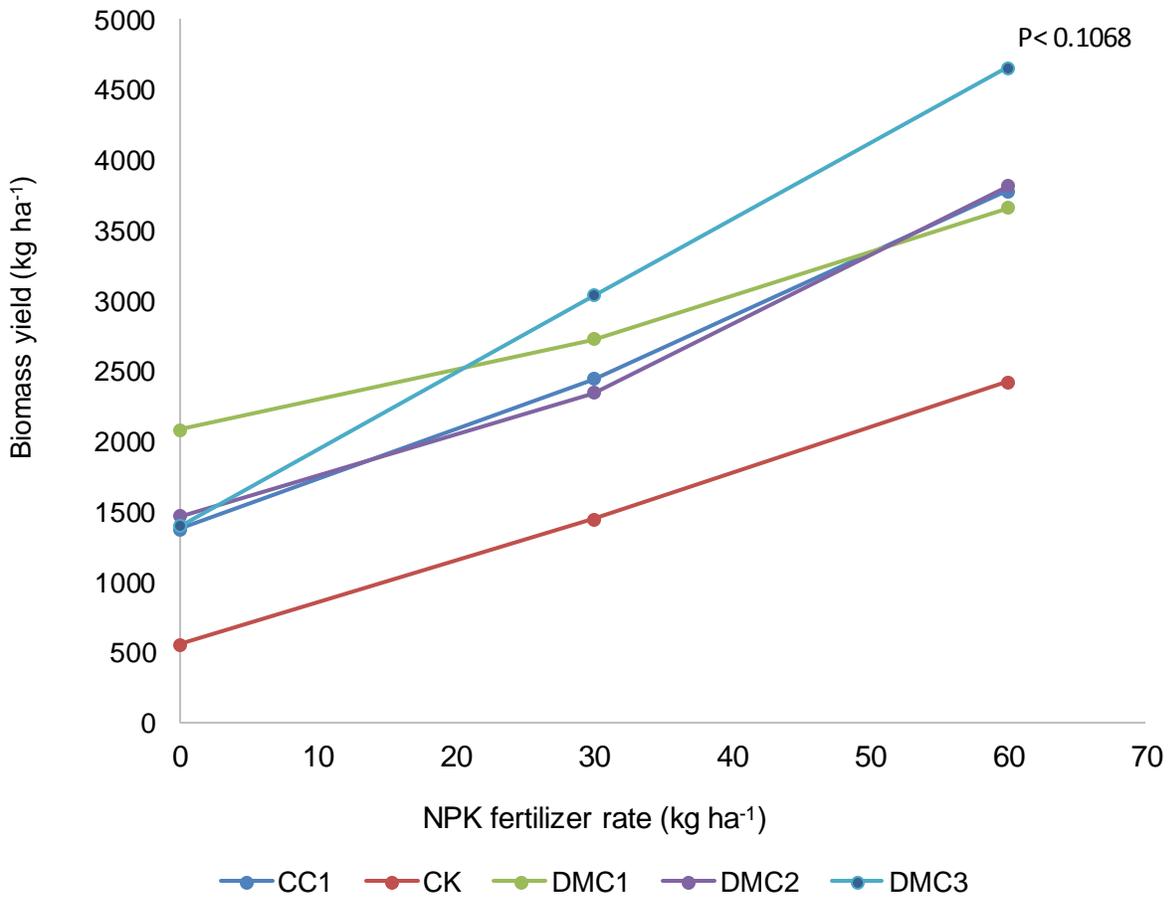DMC3 = Maize + Cowpea

Figure 3.4. Plant biomass yield as affected by the interactive effect of cropping systems and fertilizer in 2012 at Nyankpala, Ghana.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
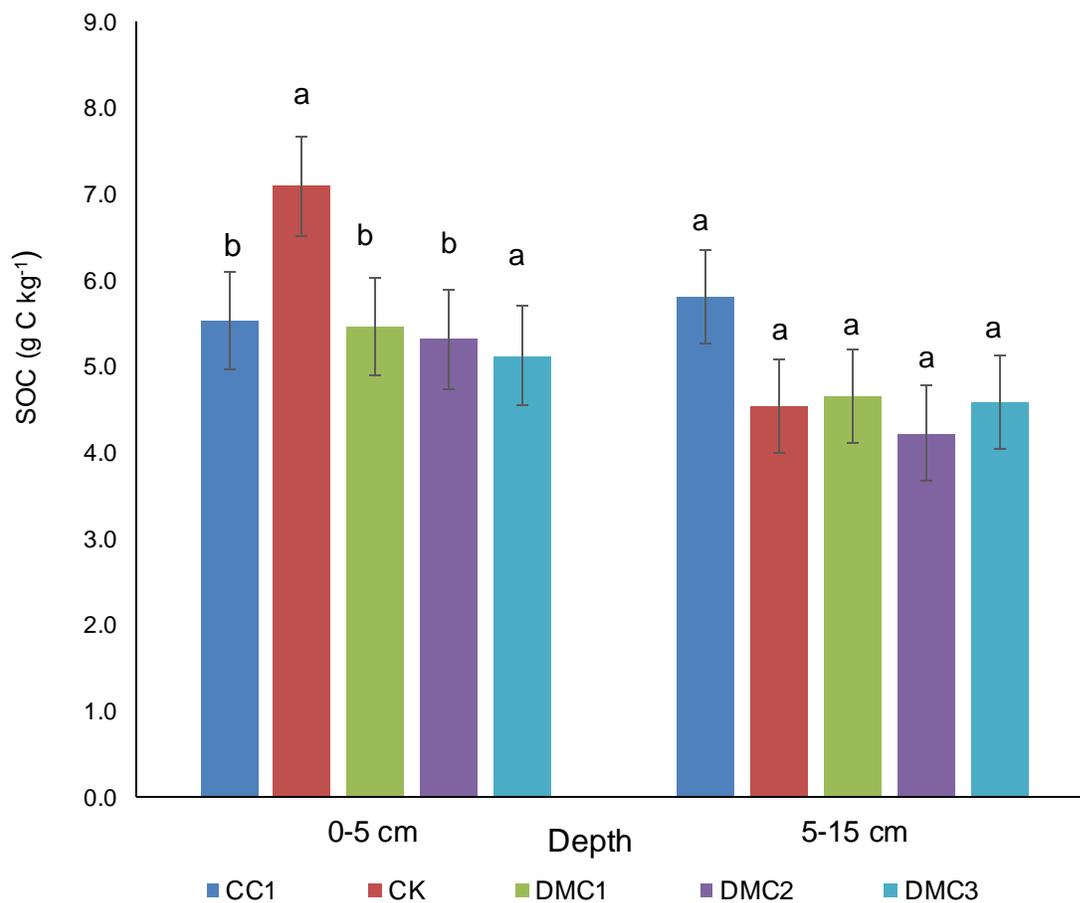DMC3 = Maize + Cowpea

Figure 3.5. Soil organic carbon as affected by cropping systems at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea

Figure 3.6. Soil organic carbon as affected by fertilizer at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea

Figure 3.7. Relationship between soil organic carbon (SOC) and fertilizer at 0-5 cm at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
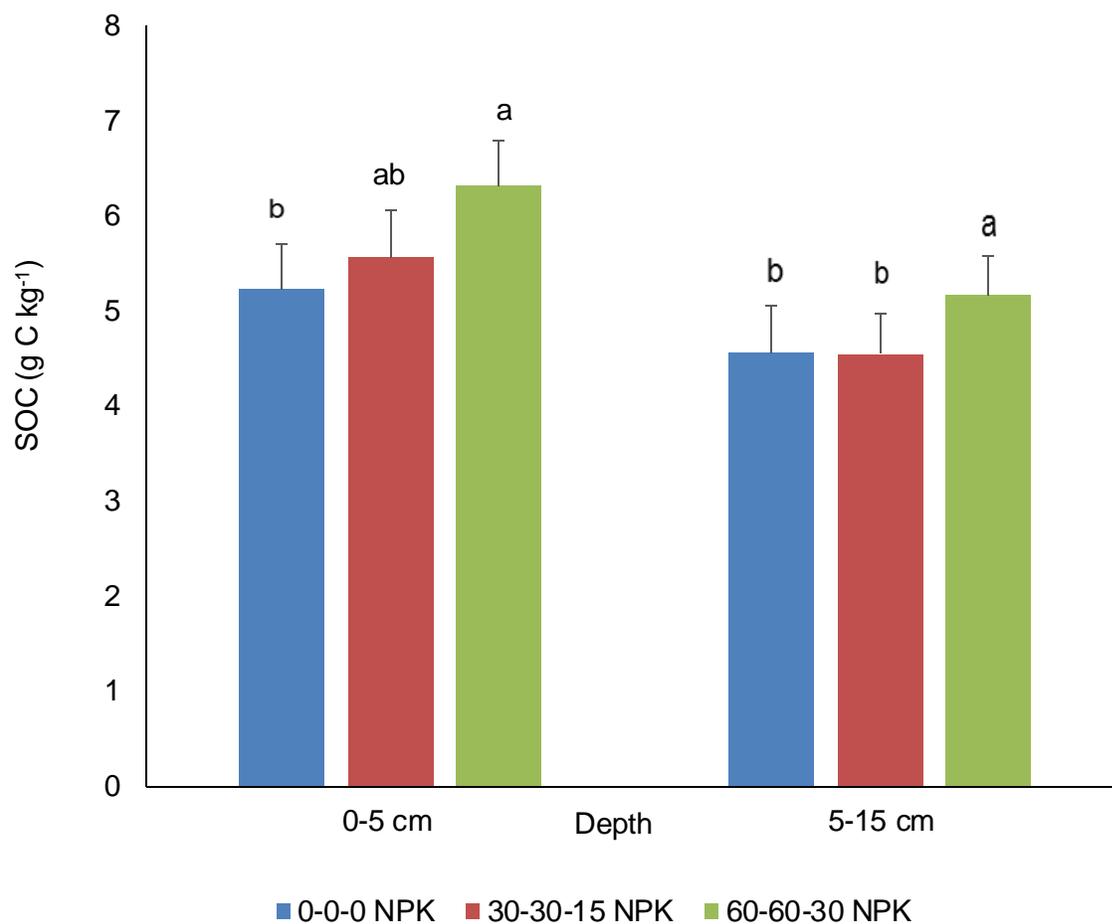DMC3 = Maize + Cowpea

Figure 3.8. Relationship between soil organic carbon (SOC) and fertilizer at 5-15 cm at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea

Figure 3.9. Soil total nitrogen as affected by cropping systems at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
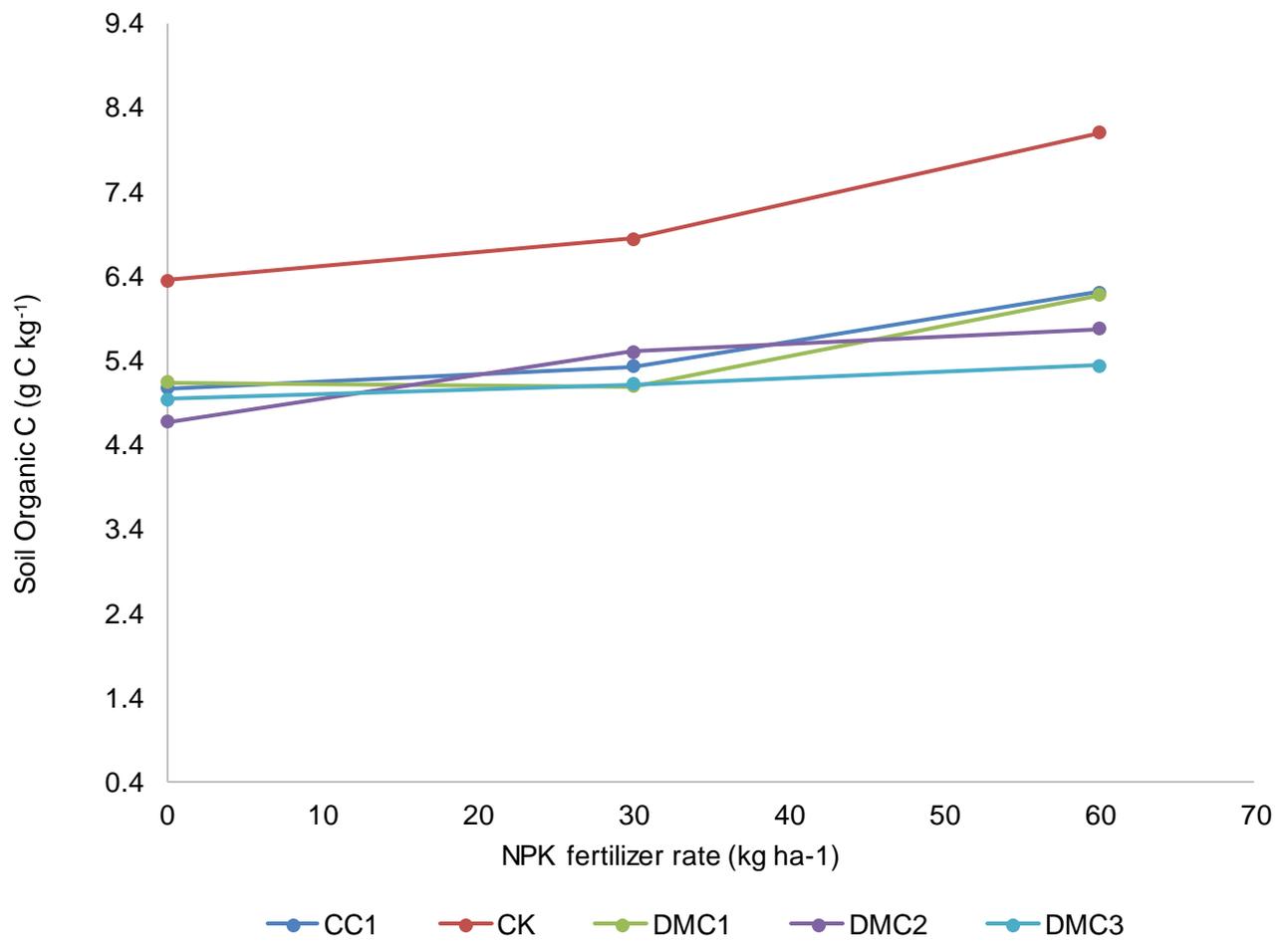DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea

Figure 3.10. Soil total nitrogen as affected by cropping systems at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species

CK = Maize (check)

DMC1 = Maize + Stylosanthese

DMC2 = Maize + Black Dolichos *lab lab*
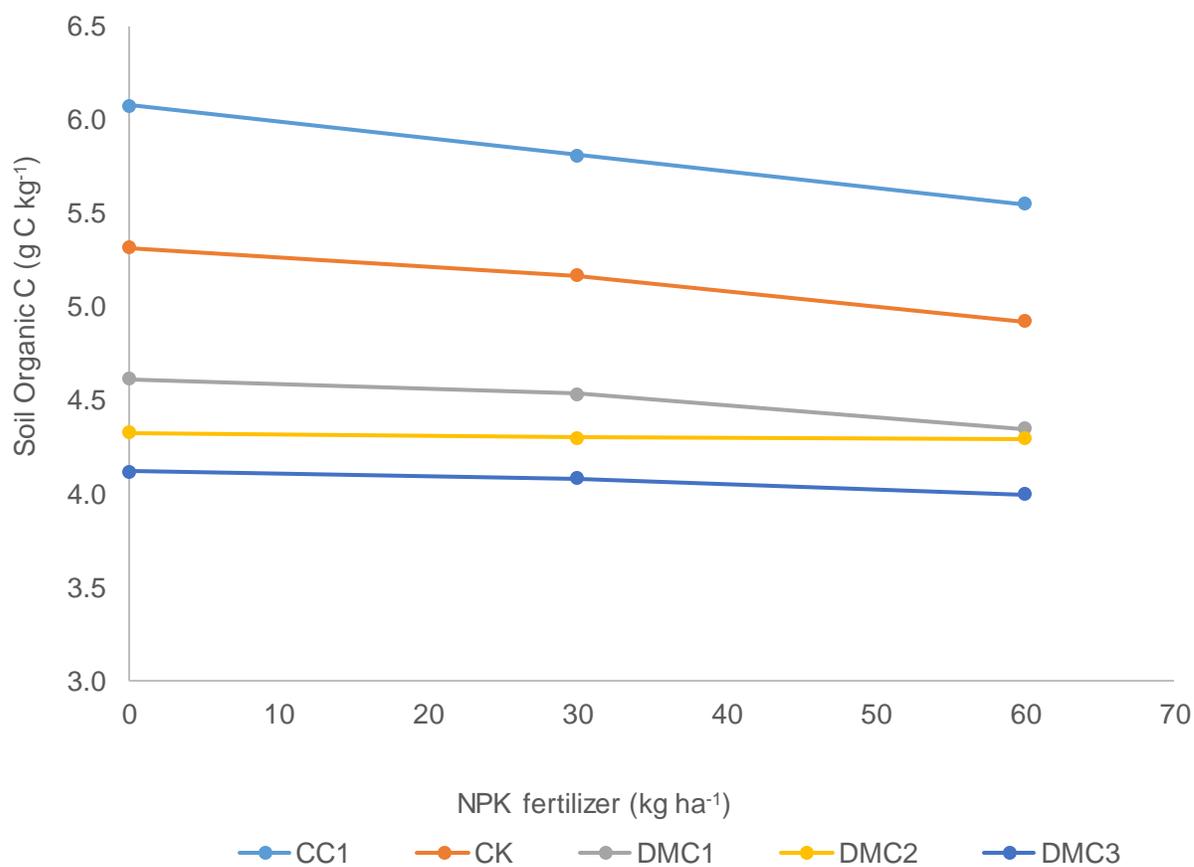
DMC3 = Maize + Cowpea
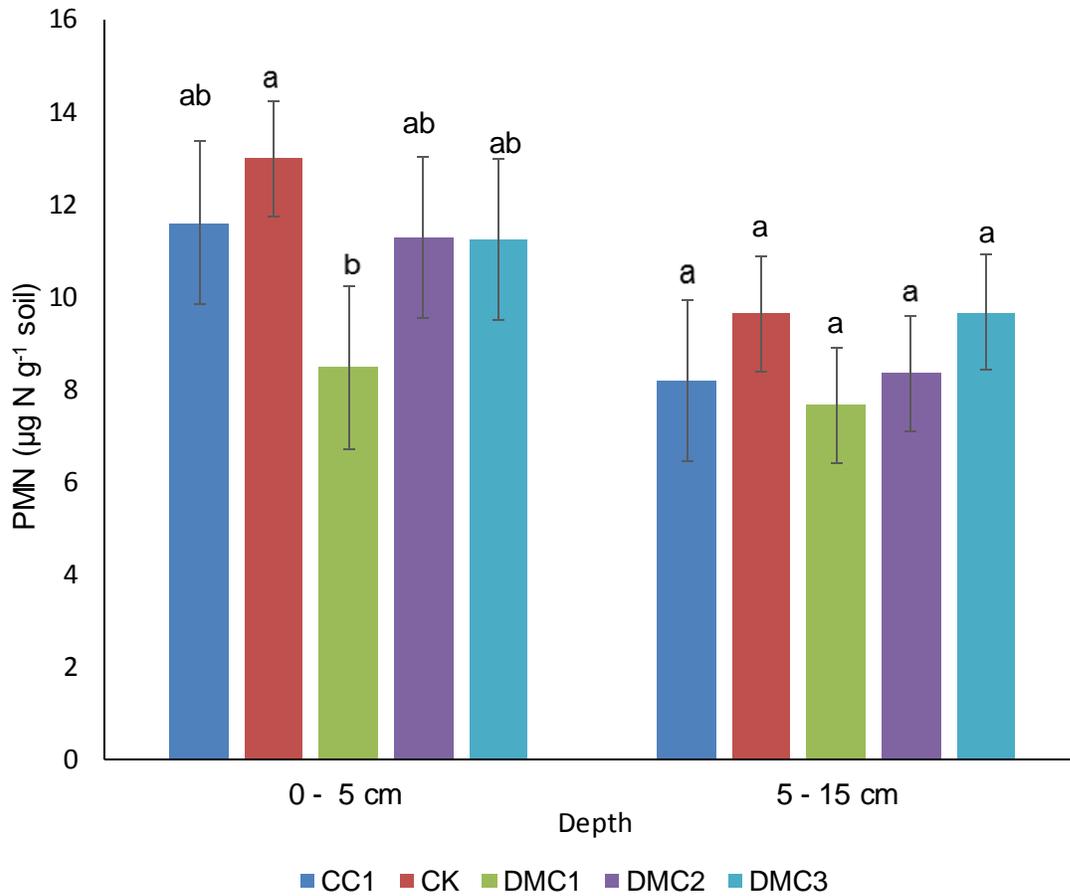
Figure 3.11. Potentially mineralizable N (PMN) as affected by cropping systems at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea
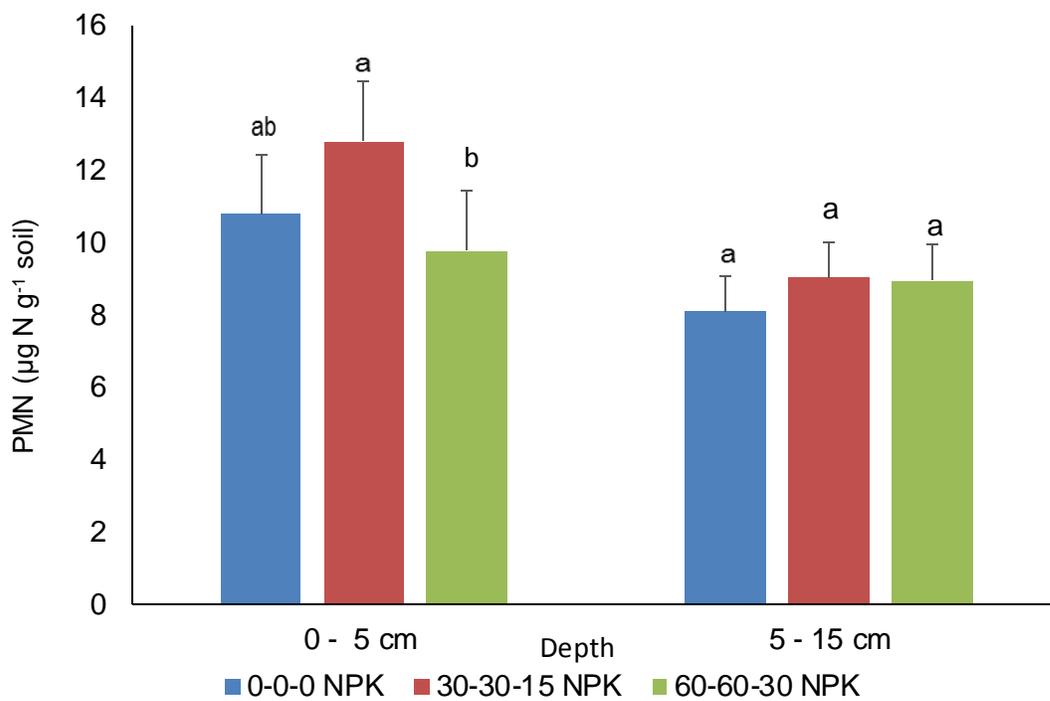
Figure 3.12. Potentially mineralizable N (PMN) as affected by fertilizer at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species

CK = Maize (check)

DMC1 = Maize + Stylosanthese

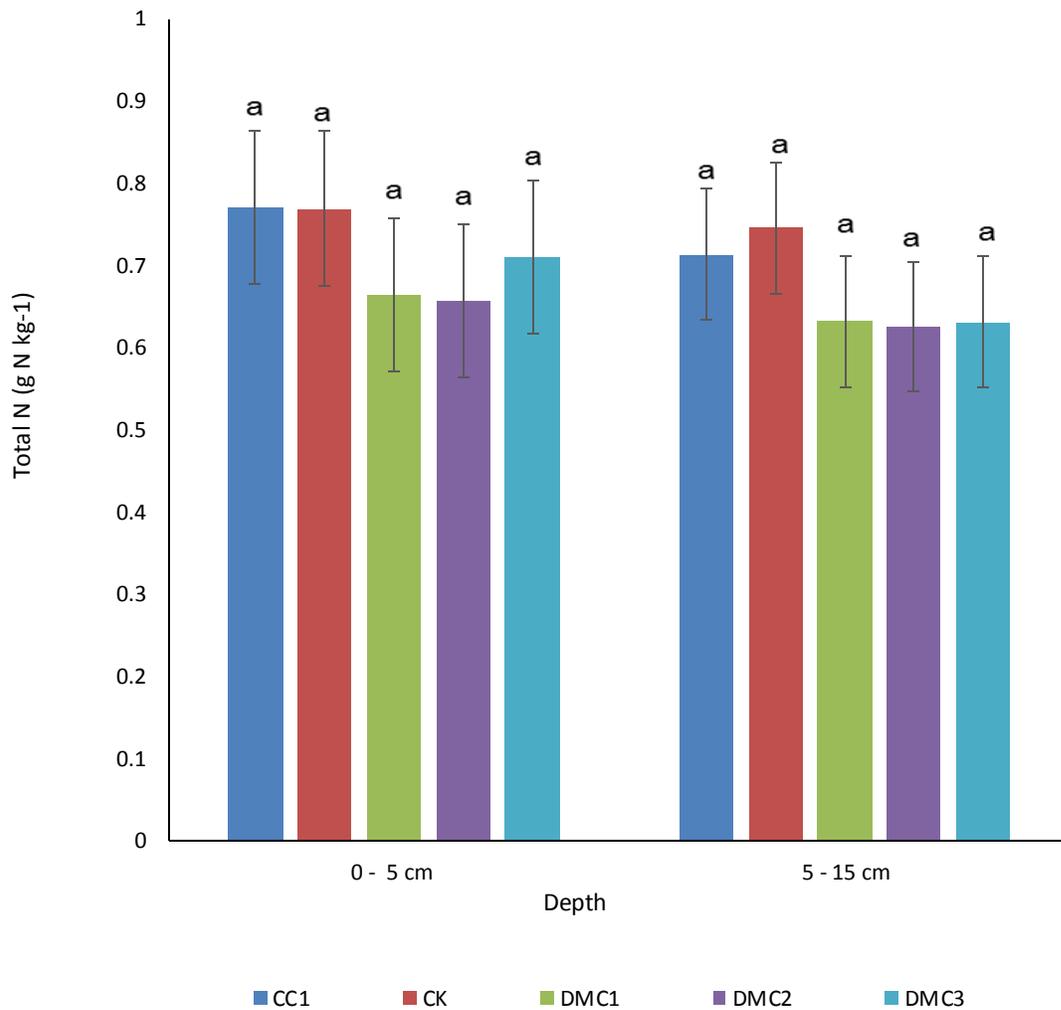DMC2 = Maize + Black Dolichos *lab lab*

DMC3 = Maize + Cowpea

Figure 3.13. Soil pH as affected by cropping systems at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species

CK = Maize (check)
DMC1 = Maize + Stylosanthese
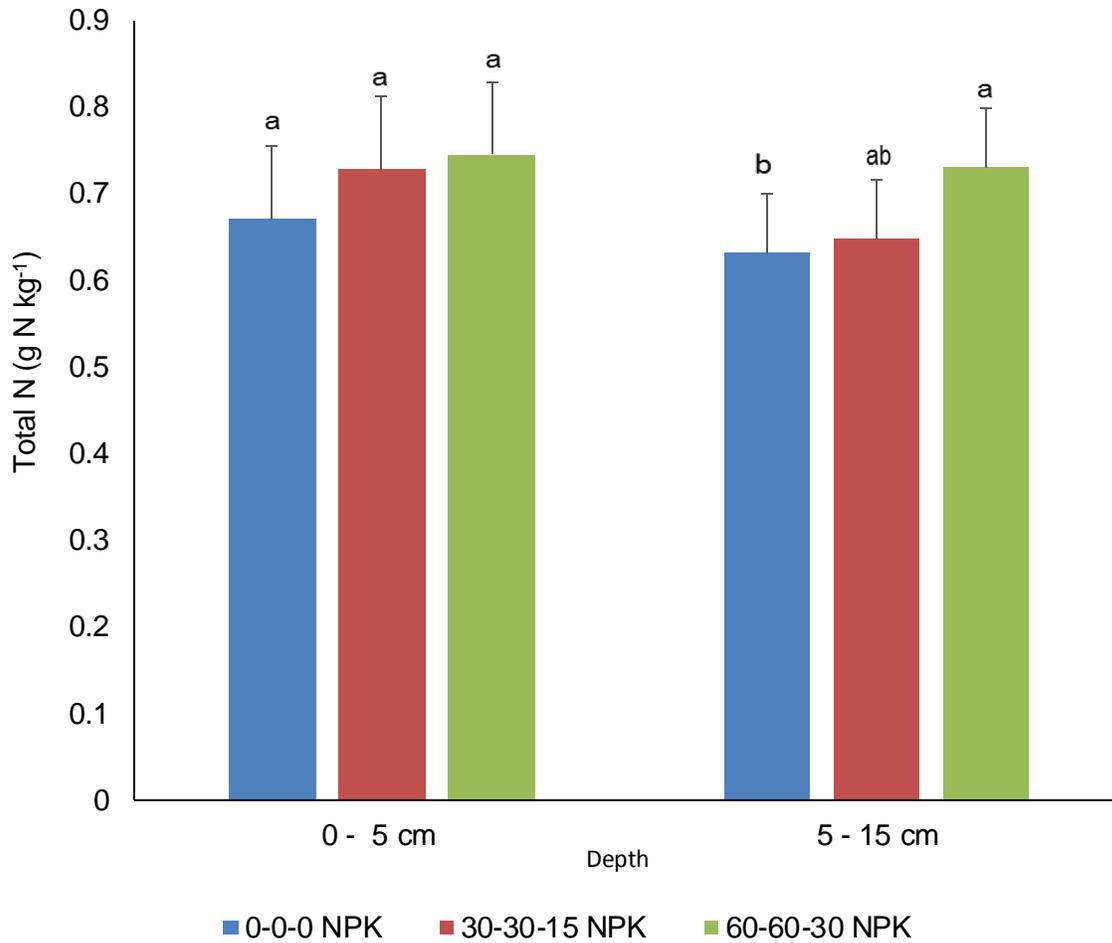DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea

Figure 3.14. Soil pH as affected by fertilizer at different depths at Nyankpala in Ghana, 2014.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea

Table 3.1. Physical and chemical properties of the 0-20 cm soil at trial site before trial was initiated in 2011 at Nyankpala, Ghana.

Soil properties	0-20 cm
pH (CaCl$_2$)	6
pH (water)	5.8
Organic carbon (%)	0.43
Total N (%)	0.04
Available P (mg kg^{-1})	11
Exchangeable cation	
K (cmol kg^{-1})	0.24
Ca (cmol kg^{-1})	2.3
Mg (cmol kg^{-1})	0.5
Na (cmol kg^{-1})	0.11
(Al+H) (cmol kg^{-1})	1.1
ECEC (cmol kg^{-1})	4.5
Base saturation (%)	74.8
Particle size	
Sand (%)	75.5
Silt (%)	12.5
Clay (%)	12
Moisture content (%)	0.2
Bulk density (g cm^{-3})	1.45

(Baba et al., 2013)

Table 3.2. The effect of cropping systems and fertilizer on soil microbial respiration (SMR), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) with respect to different depths at Nyankpala in Ghana, 2014.

	Depth (cm)					
	0 - 5			5 - 15		
	SMR	MBC	MBN	SMR	MBC	MBN
	μg C g^{-1} soil 10 d^{-1}	μg C g^{-1} soil		μg C g^{-1} soil 10 d^{-1}	μg C g^{-1} soil	
Cropping systems						
CC1	107	141	12	99	148	19
CK	111	203	11	99	128	19
DMC1	111	183	15	97	131	20
DMC2	105	185	9	97	118	22
DMC3	102	147	13	95	109	22
LSD (P< 0.05)	NS	NS	NS	NS	NS	NS
NPK Fertilizer (kg ha^{-1})						
0-0-0	102 b	170	6 b	98	97	21
30-30-15	111 a	184	17 a	101	101	21
60-60-30	108 ab	161	13 a	93	93	20
LSD (P< 0.05)		NS		NS	NS	NS
		P > F			P > F	
Cropping System (C)	0.160	0.226	0.762	0.952	0.51	0.625
Fertilizer (F)	0.0336	0.425	0.001	0.251	0.754	0.71

Means in column with the same letter(s) are not significantly different P< 0.05.

NS = Not significantly different.

CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
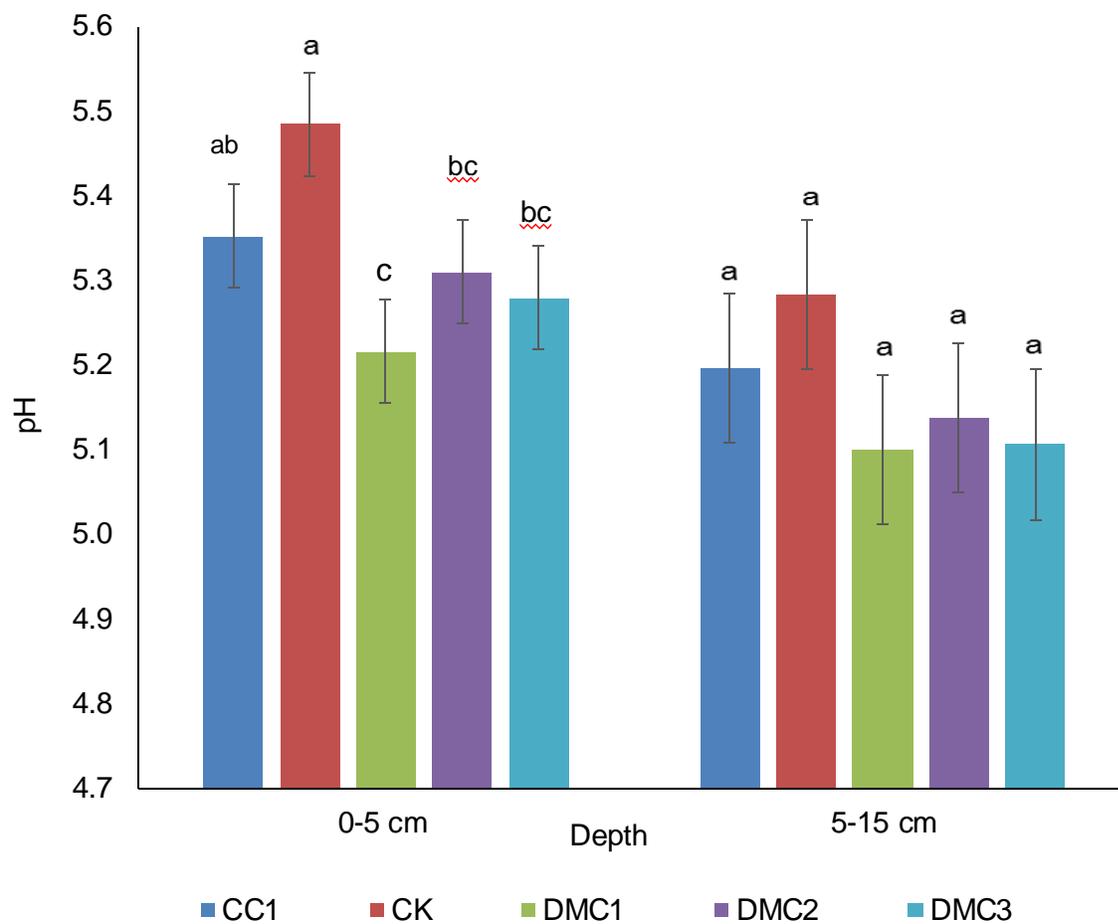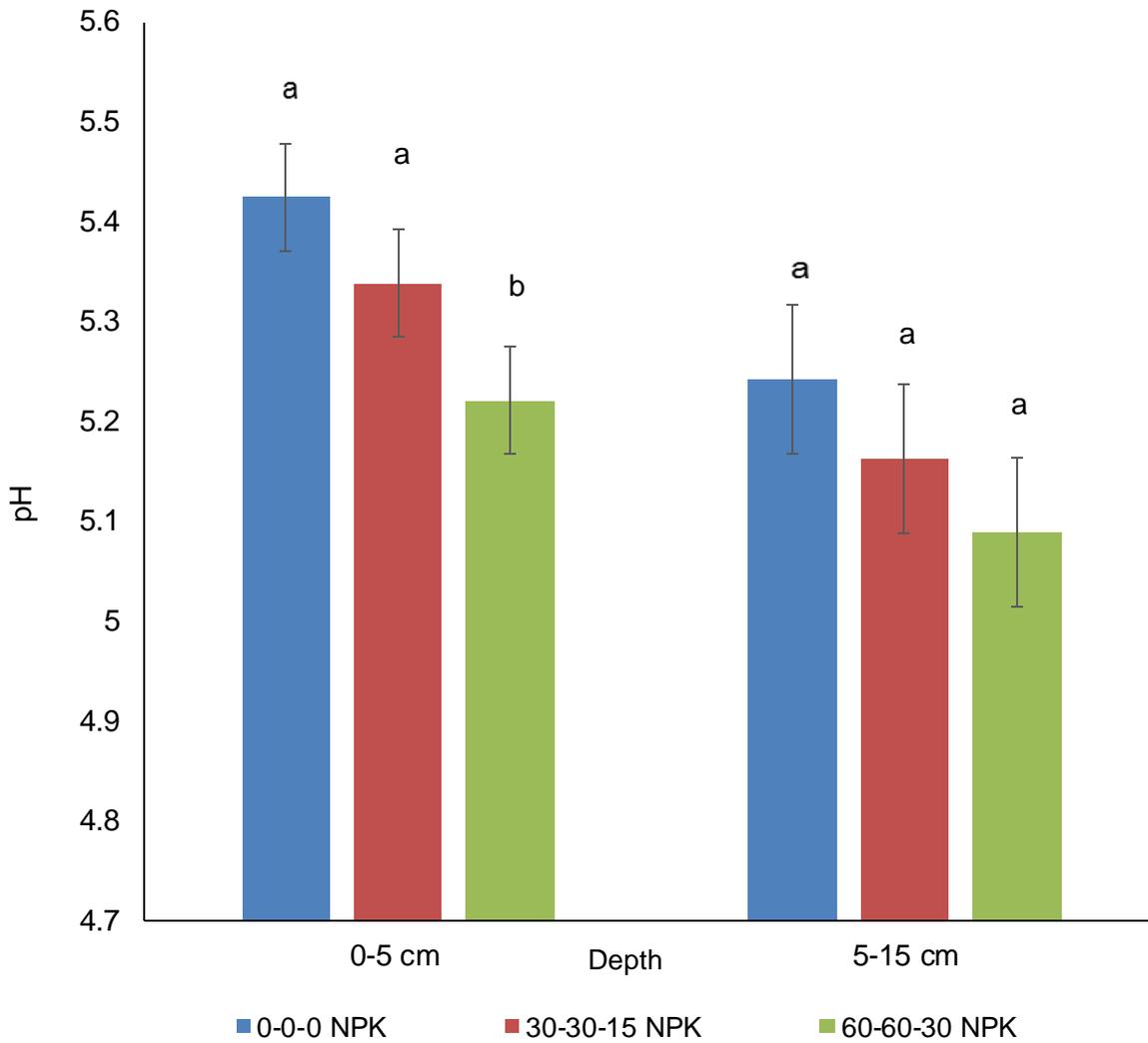DMC3 = Maize + Cowpea

Table 3.3. The interactive effect of cropping systems and fertilizer on soil microbial respiration microbial biomass carbon (MBC) and soil organic carbon (SOC) at different depths at Nyankpala in Ghana in 2014.

Interactive effect	Depth (cm)					
	0 – 5			5 -15		
	Soil respiration	MBC	SOC	Soil respiration	MBC	SOC
	µg C g^{-1} soil^{-1} 10 d^{-1}	µg C g^{-1} soil	g C kg^{-1}	µg C g^{-1} soil^{-1} 10 d^{-1}	µg C g^{-1} soil	g C kg^{-1}
CC1 + 0-0-0 NPK	100	158	5.0	99	136	6.1
CC1 + 30-30-15 NPK	108	125	5.3	107	153	5.8
CC1+ 60-60-30 NPK	114	139	6.2	98	153	5.6
CK + 0-0-0 NPK	109	170	6.3	107	126	5.3
CK+ 30-30-15 NPK	113	258	6.8	92	118	5.2
CK+ 60-60-30 NPK	110	180	8.1	94	141	4.9
DMC1+ 0-0-0 NPK	106	192	5.1	101	130	4.6
DMC1+ 30-30-15 NPK	113	208	5.1	95	136	4.5
DMC1+ 60-60-30 NPK	113	151	6.2	91	127	4.3
DMC2+ 0-0-0 NPK	97	193	4.7	89	123	4.3
DMC2+ 30-30-15 NPK	109	180	5.5	94	100	4.3
DMC2+ 60-60-30 NPK	107	181	5.8	100	131	4.3
DMC3 + 0-0-0 NPK	96	139	4.9	97	93	4.1
DMC3 + 30-30-15 NPK	111	149	5.1	100	125	4.1
DMC3 + 60-60-30 NPK	98	154	5.0	93	108	6.1
LSD (P< 0.05)	NS	NS	NS	NS	NS	NS
	P > F			P > F		
C X F	0.7698	0.469	0.988	0.701	0.943	0.753

NS =Not significantly different.
CC1 = Mixed strand of cover crop species. CK = Maize (check). DMC1 = Maize + Stylosanthese. DMC2 = Maize + Black Dolichos *lab lab* . DMC3 = Maize + Cowpea

Table 3.4. The interactive effect of cropping systems and fertilizer on microbial biomass nitrogen (MBN), potentially mineralizable nitrogen (PMN) and soil total nitrogen (Total N) at different depths at Nyankpala in Ghana, 2014.

	Depth (cm)					
	0 – 5			5 – 15		
Interactive effect	MBN	PMN	Total N	MBN	PMN	Total N
	μg N g^{-1} soil		g N kg^{-1}	μg N g^{-1} soil		g N kg^{-1}
CC1 + 0-0-0 NPK	11	9	0.68	18 bcd	8	0.72
CC1 + 30-30-15 NPK	11	14	0.79	19 bcd	9	0.69
CC1+ 60-60-30 NPK	13	12	0.84	19 bcd	7	0.73
CK + 0-0-0 NPK	5	13	0.73	13 d	9	0.71
CK + 30-30-15 NPK	21	14	0.79	23 abc	11	0.8
CK+ 60-60-30 NPK	7	12	0.79	22 abc	9	0.73
DMC1+ 0-0-0 NPK	5	9	0.64	23 abc	6	0.58
DMC1+ 30-30-15 NPK	17	11	0.65	22 abc	10	0.62
DMC1+ 60-60-30 NPK	22	6	0.71	16 cd	7	0.7
DMC2+ 0-0-0 NPK	4	13	0.60	20 bcd	8	0.57
DMC2+ 30-30-15 NPK	14	11	0.70	22 abc	8	0.60
DMC2+ 60-60-30 NPK	10	10	0.65	25 ab	9	0.64
DMC3 + 0-0-0 NPK	7	11	0.71	29 a	9	0.66
DMC3 + 30-30-15 NPK	19	15	0.70	22 abc	8	0.58
DMC3 + 60-60-30 NPK	12	9	0.75	16 cd	12	0.49
LSD (P< 0.05)	NS	NS	NS		NS	NS
	P > F			P > F		
C X F	0.121	0.547	0.914	0.06	0.250	0.148

Means in column with the same letter(s) are not significantly different at P< 0.05. .

NS= Not significantly different C = cropping systems. F =Fertilizer

CC1 = Mixed strand of cover crop species. CK = Maize (check). DMC1 = Maize + Stylosanthese. DMC2 = Maize + Black Dolichos *lab lab* . DMC3 = Maize + Cowpea

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Table 3.5. The effect of cropping systems and fertilizer on soil inorganic nitrogen (N), phosphorus (P) and potassium (K) at different depths at Nyankpala in Ghana, 2014.

	Depth (cm)					
	0 – 5			5 – 15		
	N	P	K	N	P	K
Cropping systems	mg kg^1 of soil					
CC1	25	7.3	82 b	14	4.6	44
CK	23	7.2	112 a	14	3.0	54
DMC1	26	5.2	87 b	17	3.6	49
DMC2	21	7.1	100 ab	15	3.9	49
DMC3	22	6.2	91 ab	14	4.5	54
LSD (P< 0.05)	NS	NS		NS	NS	NS
NPK Fertilizer (kg ha^{-1})						
0-0-0	20 b	5.5 b	91	12 b	3.6	46 b
30-30-15	22 ab	7.7 a	93	13 b	4.2	47 b
60-60-30	28 a	6.6 ab	100	19 a	4.0	56 a
LSD (P< 0.05)			NS		NS	
	P > F			P > F		
Cropping System (CS)	0.717	0.372	0.010	0.644	0.313	0.450
Fertilizer (F)	0.019	0.025	0.390	0.007	0.625	0.065
CS x F	0.712	0.119	0.846	0.724	0.391	0.320

Means in column with the same letter(s) are not significantly different.

NS = Not significantly different.
CC1 = Mixed strand of cover crop species
CK = Maize (check)
DMC1 = Maize + Stylosanthese
DMC2 = Maize + Black Dolichos *lab lab*
DMC3 = Maize + Cowpea

Table 3.6. The interactive effect of cropping systems and fertilizer on inorganic nitrogen (N), phosphorus (P) and potassium (K) and pH at different depths at Nyankpala in Ghana, 2014.

	Depth (cm)							
	0 − 5				5 -1 5			
Interactive effect	N	P	K	pH H$_2$0	N	P	K	pH
	mg^{-1} kg of soil			H$_2$O (1:1)	mg^{-1} kg of soil			H$_2$O (1:1)
CC1 + 0-0-0 NPK	24	4.7	77	5.4	13	3.5	44	5.3
CC1 + 30-30-15 NPK	20	8.7	76	5.4	14	6.3	44	5.1
CC1+ 60-60-30 NPK	30	8.3	93	5.3	14	4.0	44	5.2
CK + 0-0-0 NPK	16	4.5	119	5.7	11	3.0	54	5.4
CK + 30-30-15 NPK	26	7.5	106	5.5	12	3.2	52	5.3
CK + 60-60-30 NPK	26	9.7	112	5.3	18	2.6	57	5.2
DMC1+ 0-0-0 NPK	24	5.6	85	5.3	12	2.9	45	5.2
DMC1+ 30-30-15 NPK	24	6.3	86	5.3	15	3.7	48	5.2
DMC1+ 60-60-30 NPK	30	3.7	90	5.1	24	4.1	53	5.0
DMC2+ 0-0-0 NPK	15	6.4	91	5.4	16	4.6	47	5.1
DMC2+ 30-30-15 NPK	20	9.1	108	5.3	11	2.6	48	5.2
DMC2+ 60-60-30 NPK	29	5.6	102	5.2	18	4.5	51	5.0
DMC3 + 0-0-0 NPK	19	6.0	81	5.3	11	4.1	40	5.2
DMC3 + 30-30-15 NPK	21	6.9	90	5.2	11	5.1	45	5.1
DMC3 + 60-60-30 NPK	24	5.8	102	5.2	20	4.4	76	5.1
LSD (P< 0.05)	NS	NS	NS	NS	NS	NS	NS	NS
	P > F				P > F			
C X F	0.712	0.119	0.846	0.297	0.7241	0.3907	0.320	0.983

NS= Not significantly different at P< 0.05. C = cropping systems. F =Fertilizer
CC1 = Mixed strand of cover crop species. CK = Maize (check). DMC1 = Maize + Stylosanthese. DMC2 = Maize + Black Dolichos *lab lab* . DMC3 = Maize + Cowpea.

Table 3.7. The effect of cropping systems and fertilizer on soil calcium and magnesium with respect to different depths at Nyankpala in Ghana, 2014.

	Depth (cm)			
	0 - 5		5-15	
	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}
	mg kg^{-1} of soil			
Cropping **systems**				
CC1	441	90	405	73
CK	393	111	348	90
DMC1	361	92	337	75
DMC2	344	88	333	75
DMC3	310	90	326	74
LSD (P< 0.05)	NS	NS	NS	NS
NPK Fertilizer (kg ha^{-1})				
0-0-0	374	96	355	77
30-30-15	369	97	347	76
60-60-30	366	90	346	79
LSD (P< 0.05)	NS	NS	NS	NS
	P > F		P > F	
Cropping System (C)	0.115	0.146	0.311	0.306
Fertilizer (F)	0.859	0.225	0.792	0.526
C X F	0.561	0.879	0.993	0.607

NS= Not significantly different

CC1 = Mixed strand of cover crop species

CK = Maize (check)

DMC1 = Maize + Stylosanthese

DMC2 = Maize + Black Dolichos *lab lab*

DMC3 = Maize + Cowpea

Table 3.8. The interactive effect of cropping systems and fertilizer calcium (Ca^{2+}) and magnesium (Mg^{2+}) at different depths at Nyankpala in Ghana, 2014.

	Depth (cm)			
	0 - 5		5 -15	
	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}
	mg^{-1} kg of soil			
CC1 + 0-0-0 NPK	377	90	352	74
CC1 + 30-30-15 NPK	398	90	335	72
CC1+ 60-60-30 NPK	403	88	355	72
CK + 0-0-0 NPK	453	118	407	91
CK + 30-30-15 NPK	472	119	408	91
CK + 60-60-30 NPK	397	97	401	88
DMC1+ 0-0-0 NPK	350	91	317	74
DMC1+ 30-30-15 NPK	353	93	331	73
DMC1+ 60-60-30 NPK	380	91	350	78
DMC2+ 0-0-0 NPK	309	88	325	75
DMC2+ 30-30-15 NPK	302	90	328	76
DMC2+ 60-60-30 NPK	319	85	324	75
DMC3 + 0-0-0 NPK	341	91	334	73
DMC3 + 30-30-15 NPK	346	92	329	69
DMC3 + 60-60-30 NPK	344	85	348	81
LSD (P< 0.05)	NS	NS	NS	NS
	P > F		P > F	
C X F	0.561	0.879	0.993	0.607

NS=Not significantly different.

CC1 = Mixed strand of cover crop species. CK = Maize (check). DMC1 = Maize + Stylosanthese. DMC2 = Maize + Black Dolichos *lab lab* . DMC3 = Maize + Cowpea

Chapter 4 - General Summary

Adoption of sustainable soil management practices such as no–tillage, cover crops and direct-seed mulch cropping systems can improve soil health. Research was undertaken to determine: (1) the effect of summer and winter cover crops on soil health in wheat grain sorghum soybean rotation in Kansas, USA; and (2) the effect of direct seed mulch–based cropping (DMC) systems on soil health in Nyankpala, Ghana. Soil health indicators assessed included: potentially mineralizable N (PMN), microbial biomass C and N (MBC & MBN), soil organic C (SOC) and Total N (TN), PLFA, bulk density, aggregate stability, soil pH, soil inorganic N, P, K, Ca, and Mg.

Cover crops and DMC cropping systems had no significant impact on most of the soil health indicators. The key observations were:

1. In Ghana, DMC cropping systems produced greater biomass, which was the driving force for soil health improvement. However, the increased biomass did not affect the biological indicators: PMN MBC, MBN, SMR and the chemical indicators: SOC, TN, inorganic N, P, K, Ca, and Mg. Similarly nutrient management resulted in greater biomass and affected both biological and chemical indicators assessed except MBC, Ca, and Mg. The response of these indicators to nutrient management may be due to the low levels of soil fertility. The non-response or negative of health indicators to DMC cropping systems was likely due to low soil pH and inadequate plant biomass inputs to increase SOC. Another reason for the lack of response may be due to the short time frame of the experiment, time of soil sampling, and weather.

2. In Kansas, cover crops did not increased microbial biomass, and aggregate stability as hypothesized. However, cover crops weakly affected MBN, SMR and microbial community

structure (F:B ratio, actinomycetes, and total PLFA). These changes may be due to increased plant residues with the cover crops. However the effect of the cover crops on soil health indicators were not consistent. Nitrogen management history during the previous sorghum phase of the rotation had no impact on the soil health indicators. The non-responsiveness of the soil health indicators to cover crops was surprising since the trial was established for 8 yrs. Perhaps the cover crops did not produce enough biomass to drive the system. Since we cannot assigned a particular reason for non-effect of sustainable cropping systems to improve soil health we recommend that further research is needed. Future research should include more detailed temporal sampling to closely match plant and weather cycles. Alternative indicators should be examined for their sensitivity to soil and crop management.
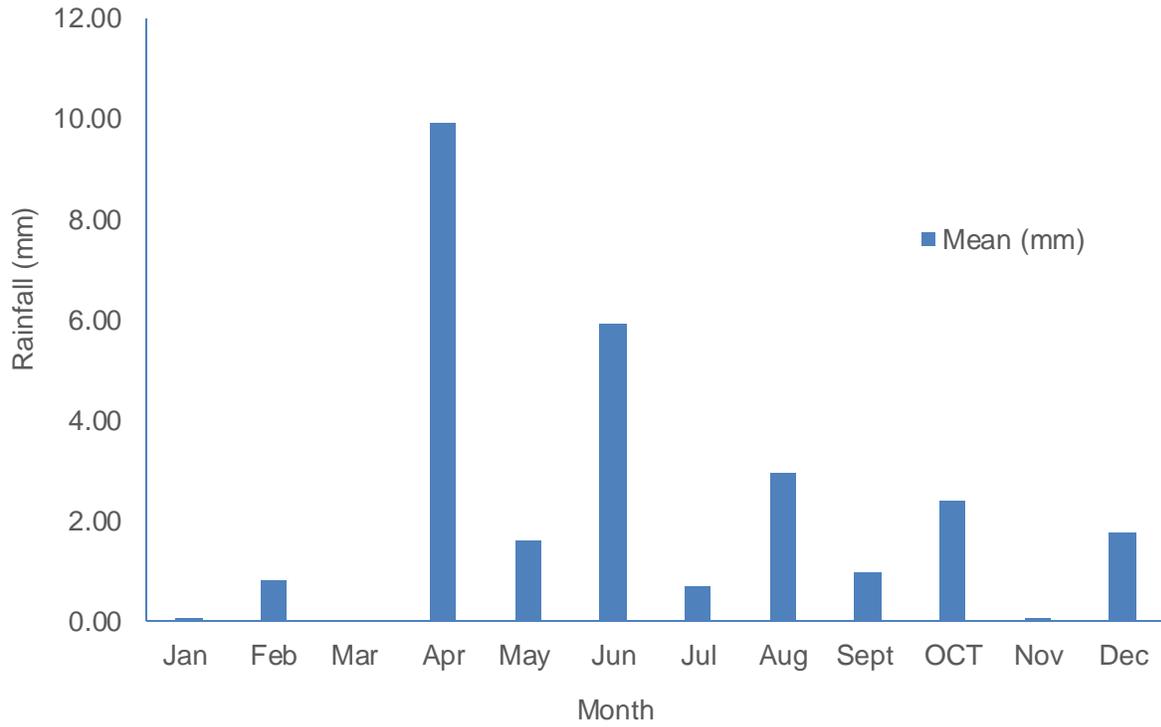
Appendix A - List of Figures for Chapter 2

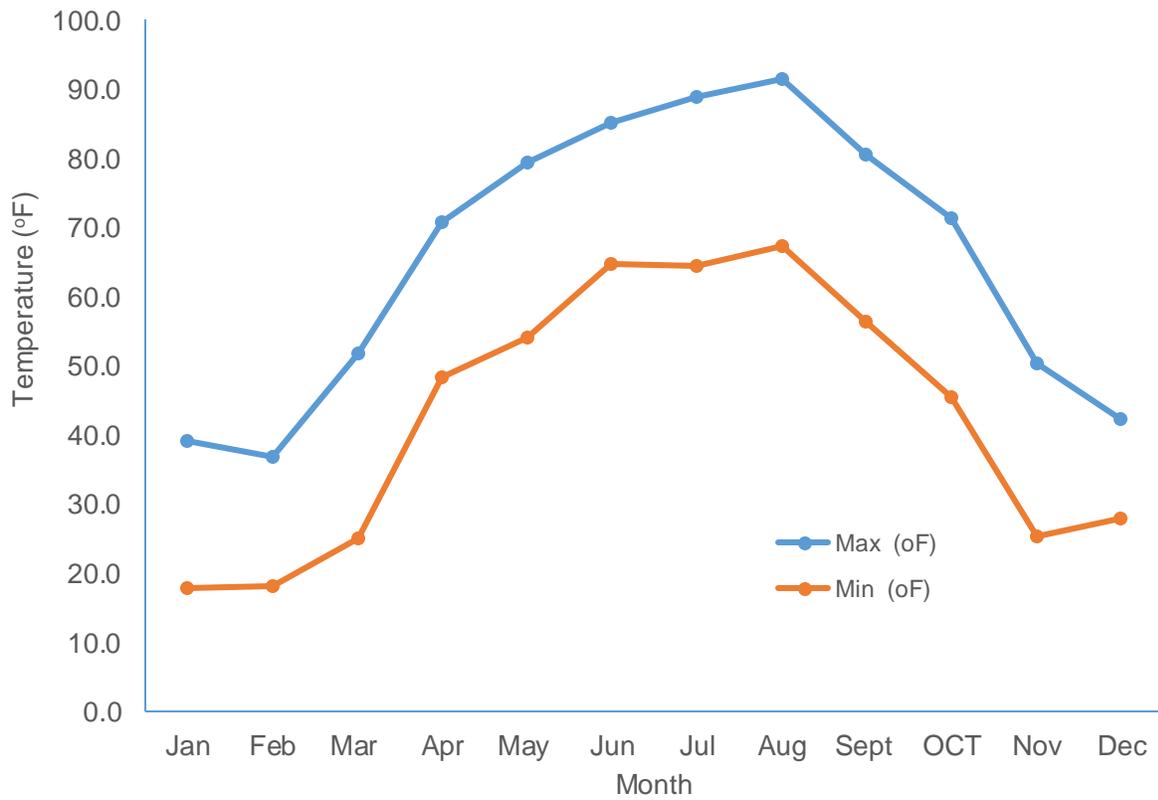Figure A.1. Average monthly rainfall distribution for the year 2014 in Ashland Bottoms, Kansas

Figure A.2. Average monthly rainfall distribution for the year 2014 in Ashland bottoms, Kansas.
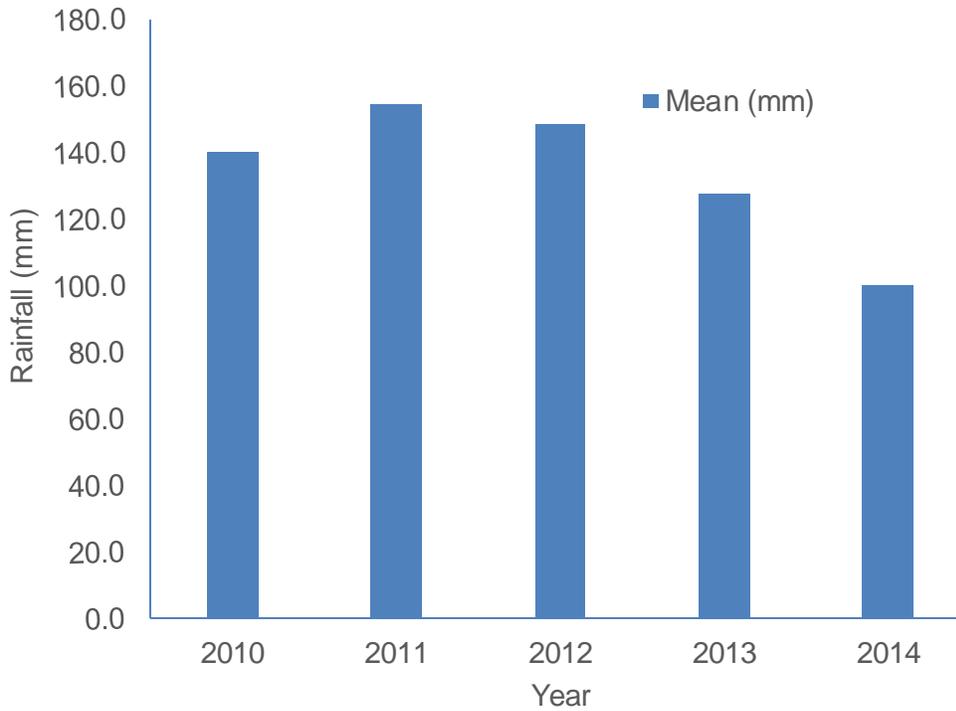
Appendix B - List of figures for chapter 3

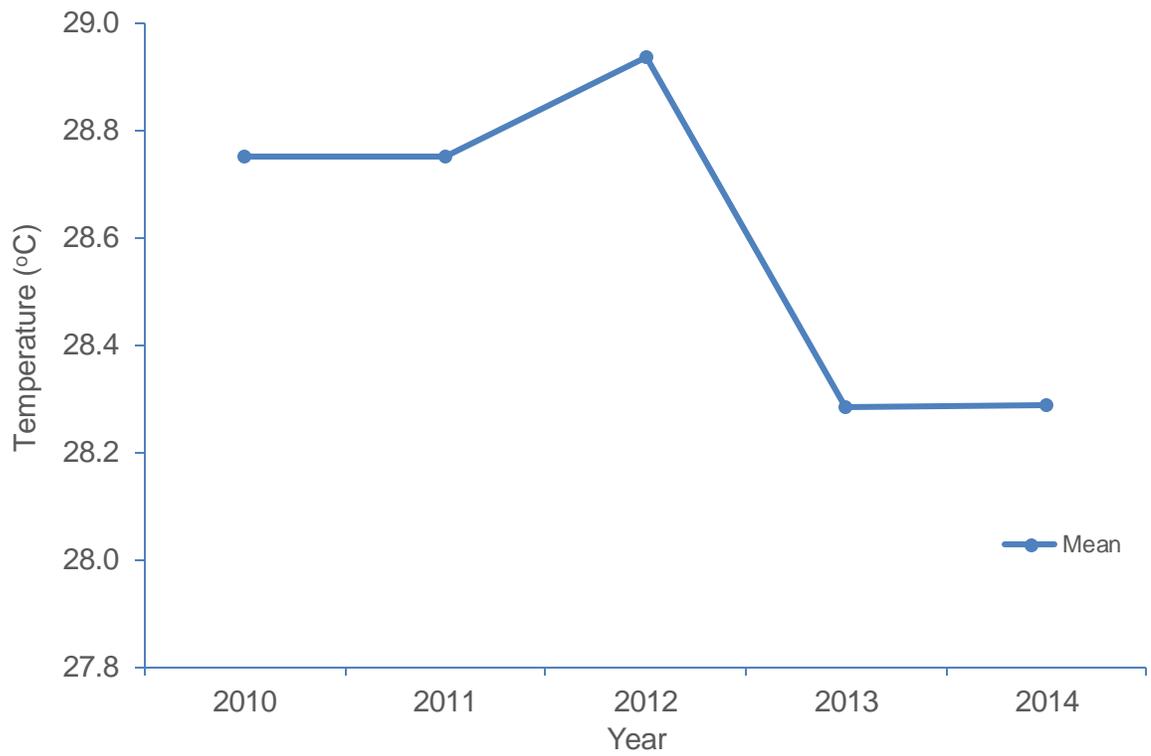Figure B.1. Five (5) years average annual rainfall distribution in Nyankpala, Ghana.

Figure B.2. Five (5) years average annual temperature in Nyankpala, Ghana.