SOIL ORGANIC CARBON DYNAMICS AND MYCORRHIZAL FUNGAL DIVERSITY IN CONTRASTING AGROECOSYTEMS

By

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ABSTRACT

SOIL ORGANIC CARBON DYNAMICS AND MYCORRHIZAL FUNGAL DIVERSITY IN CONTRASTING AGROECOSYTEMS

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Maintenance and improvement of soil quality is critical to sustaining agricultural productivity and environmental quality. Soil organic carbon (SOC) and Arbuscular Mycorrhizal Fungi (AMF) are among key soil quality and agronomic sustainability indicators. AMF are involved in nutrient transfers and C sequestration, relevant in the global carbon cycle and greenhouse gas abatement. Conventional agriculture adversely affects SOC, AMF and the environment, yet little is known on the impact of alternative options. In this dissertation, I examined the impact some options on selected soil quality indicators in temperate and tropical settings to address some existing research gaps.

In Chapter 1, I examined the role of nutrient management and crop diversity on SOC and aggregate stability in a 20 year field study of the Living Field Laboratory (LFL) at KBS-LTER in Michigan. I assessed responses of Integrated Fertilizer and Integrated Compost management on a diversity gradient comprising of monoculture Corn, Corn-soy rotation, Corn-soy-wheat rotation, and Corn-soy-wheat rotation with a cover crop. Management rather than diversity exerted significant influence on SOC and labile carbon (POXC) status across treatments with higher SOC and POXC levels in compost treatments. Crop diversity exerted positive influence on aggregate stability. Diverse rotations had greater aggregate stability than monocultures regardless of nutrient management system.

Crop diversity can therefore enhance soil structural stability in the long term, and compost management holds promise in ameliorating both poor soil Carbon status and soil structural stability associated with continuous corn monoculture systems.

In Chapter 2, I investigated AMF spore diversity using morphological techniques to assess abundance and diversity of AMF in the LFL. Proportions of AMF taxa varied with crop diversity. Surprisingly, nutrient management influenced soil organic matter but not AMF community composition across management systems.

In Chapter 3, I report on the influence of land use on soil bio-resources in the tropical landscape of Machinga District in Malawi, a country in Sub-Saharan Africa. I examined communities of AMF in Miombo woodlands and croplands nested within Malosa Forest Reserve. The Shannon- Weiner diversity index ($H´$) differed significantly among land use types being higher in agricultural soils than in the natural forest soils reflecting community compositional shifts among communities under study.

In Chapter 4, I report on SOC spatial distribution at landscape scale in Machinga district located in sub-Saharan Africa and evaluated SOC prediction accuracy among various interpolation techniques. SOC distribution was greatly influenced by land use type and spatial topographic attributes. Overall, mean SOC content on surface layer soils declined over a period of 2 decades. Ordinary kriging with spherical semivariogram model fitting was found to be the optimal approach for investigating SOC spatial distribution and variability in the complex landscape. The study provides important contributions to the understanding of SOC spatial distribution that can guide land management policy, carbon sequestration and climate change mitigation strategies.

To my parents, Mike Gabriel Mpeketula and Martha Maya

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KEY TO ABBREVIATIONS

LFL: Living Field Laboratory

KBS-LTER: Kellogg Biological Research Station-Long Term Ecological Research

SOC: Soil Organic Carbon

SOM: Soil Organic Matter

AMF: Arbuscular Mycorrhizal Fungi

BD: Bulk Density

WSA: Water Stable Aggregates

MWD: Mean Weight Diameter

POXC: Permanganate Oxidizable Carbon

IC: Integrated Compost

IF: Integrated Fertilizer

C: Continuous corn

CS: Corn Soy rotation

CSW: Corn Soy Wheat rotation

CSWco: Corn Soy Wheat rotation with cover crop

CHAPTER 1

INFLUENCE OF NUTRIENT MANAGEMENT AND ROTATIONAL DIVERSITY ON SOIL ORGANIC CARBON AND SOIL STRUCTURAL STABILITY IN LONG-TERM INTEGRATED MANAGEMENT SYSTEMS

ABSTRACT

Understanding processes that ameliorate cropping system productivity and sustainability is particularly important in intensively managed row crop systems. Soil organic carbon status is known to be linked to cropping system productivity, and is enhanced by nutrient management systems that incorporate compost. The effect of crop diversity, however, is not well understood in terms of soil organic carbon accrual and soil structural stabilization. We investigated the role of bio-diversification through the manipulation of crop diversity in a 20 year study of the Living Field Laboratory located at Kellogg Biological Station-Long Term Ecological Research (KBS-LTER), southwest Michigan. The treatments included continuous monoculture corn (C), Corn-soy rotation (CS), Corn-soy-wheat rotation (CSW), and a polyculture of corn-soy-wheat rotation with a cover crop (CSWco). We quantified Soil Organic Carbon (SOC), labile soil organic carbon (Permanganate Oxidizable Carbon –POXC) and water stable aggregation at 3 depths (0-5, 5-20 and 20-25 cm) to i) determine the long term response of the measures to rotational diversity in integrated compost (IC) and integrated fertilizer (IF) management systems, and ii) examine the relationship between various soil properties to structural stability of fine loamy mixed, mesic Typic Hapludalf soils at the research site. Over two decades of experimentation, our study demonstrated that management rather than diversity had profound influence on a set of soil quality indicators such as SOC and POXC. Both SOC and POXC levels were higher in plots under Integrated Compost management.

Likewise, soil structural stability was enhanced with compost-based nutrient management as evidenced by an increase (19%) in water stable macroagregates for 0-5 cm depth compared to inorganic nutrient management systems. On the other hand, the long term impact of rotational diversity was evident on different sets of soil quality measures such as soil aggregate stability. Biodiverse rotations had better aggregate stability compared to corn monocultures in both management regimes. Furthermore, improvements in soil structural stability were attainable under integrated compost management in the long term relative to integrated fertilizer and crop bio-diversification with the inclusion of cover crops was an efficient means of ameliorating poor soil structural stability associated with continuous monoculture of corn. Our findings highlight the interactions between soil physical, biological and management factors in determining the pace of trajectories in aggregate formation and stabilization and SOC accrual suggesting that although the trajectory of aggregate formation may be similar across systems, nutrient management and crop diversity gradients modulate the timing and pace of processes due to their differential effects on belowground productivity. The study provides insights on long term effects of two integrated nutrient management systems that are deployable option to conventional farming practices.

INTRODUCTION

Cropping system productivity and sustainability depend to a great extent on soil organic matter dynamics, including the turnover of labile carbon and nitrogen pools and the renewal of stabilized pools (Wander, 2004). SOC plays an important role as an indicator of soil quality and approximately 10% of the earth's total soil C (1500 Pg) (Kong et al., 2005) is stored within agricultural soils, making agricultural soils an important carbon sink. Understanding which management practices hold promise for SOC accrual and sequestration of soil C in field crop systems thus plays a crucial role in promoting agricultural sustainability, and mitigating against negative environmental impacts and anthropogenic carbon dioxide emissions.

Degradation of soil resources and aquatic environments are some of the urgent problems associated with intensive and conventional agricultural management systems. Integrated nutrient management and bio-diversification are among the limited rapidly deployable less leaky options that provide an alternative trajectory to conventional open systems which heavily rely on large doses of agro-chemical inputs (Pearson, 2007). Additionally, biodiversification has widely been regarded as a sustainability principle with positive agro ecosystems benefits such as enhanced net primary productivity, nutrient retention and resilience (Tilman et al., 1996). However, there are massive unknowns on the underlying processes by which such options improve agro ecosytem productivity and enhance agronomic sustainability. There is substantial need to elucidate mechanisms of SOC accrual and to accurately quantify underlying processes by which gains in SOC are regulated as well as identifying controls for the longevity of C pools under integrated nutrient management systems and crop diversity gradients.

According to Snapp et al., 2010, diversification of farming involves multiple temporal and spatial scales, at the landscape, community and organism levels. Studies have demonstrated that SOC accrual is directly linked to the return of fresh organic material to the soil (Rasmussen et al., 1980). Thus the inclusion of cover crops and the addition of manure may increase SOC levels in soils that are not C saturated. However, carbon gain efficiency is known to vary depending on dominant and active decomposers in the system necessitating the characterization soil microbial communities for the alternative nutrient management options and integrated farming practices.

Studies have also shown that besides C input in agro ecosystems, soil aggregate dynamics are also key determinants in SOC accrual, C sequestration and cycling (Tisdall and Oades, 1982). Soil aggregate formation and stabilization in turn influence a wide range of biological and chemical processes that regulate SOC (Tiemann and Grandy, 2015). Consequently, aggregate stability is among the key soil quality indicators that are important for informing agro ecosytem management choices. Aggregate size distribution controls soil pore space size and connectivity, which in turn influence soil microbial activity and SOC mineralization (Ananyeva et al., 2013, Tiemann and Grandy 2015). Under crop diversity gradients, variation in rooting depths, differences in amounts and quality of root exudates produced by different crops and changes in root biomass are expected to have profound effects on aggregate formation and soil carbon stabilization, yet these factors have rarely been studied along rotational crop diversity gradients in integrated management systems. Soil organic C is thought to become incorporated into and protected within aggregates in a predictable trajectory, through both physical and biological processes, moving from unstable macro-aggregates to stable micro-aggregates contained within stable macro-aggregates (Tisdall and Oades, 1982, Tiemann and Grandy 2015).

Over time, aggregates can be composed of SOC coming from different time periods, with recently added organic material located at the outer perimeter of aggregates, indicating the value of studying the proportion of stable macro-aggregates (Kadvir and Smucker, 2005). However, there is paucity in knowledge on how different C inputs sources may impact aggregate formation and SOC protection trajectories. Studies on SOC dynamics are often constrained by the long time required to discern appreciable changes in SOC. Moreover, trials designed to study the impact nutrient management have often not included integrated nutrient management systems. Consequently, there is need for improved understanding on the influence of C inputs and the complex interactions that may result from integrated nutrient management practices and rotational diversity gradients.

In this study, we examined the influence of two integrated nutrient management options namely Integrated Fertilizer and Integrated Compost and the role of rotational crop diversity on SOC and soil structural stability using a suite of soil quality indicators; namely SOC, POXC and water stable aggregate stability. We investigated the role of rotational diversity through the manipulation of crop diversity in a 20 year study located at Kellogg Biological Station, southwest Michigan. The treatments included continuous monoculture corn (C), Corn-soy rotation (CS), Corn-soy-wheat rotation (CSW), and a polyculture of corn-soy-wheat with a cover crop (CSWco). We quantified Soil Organic Carbon (SOC), labile soil organic carbon (Permanganate Oxidizable Carbon –POXC) and water stable aggregation at 3 different depths (0-5, 5-20 and 20-25 cm).

The objectives of this field study were to: i) determine the long term response of soil C and aggregation to bio-diversification in integrated compost and integrated fertilizer management systems, and ii) examine the relationship between various soil measures on structural stability of fine loamy mixed, semi active, mesic Typic Hapludalf soils of the long term trial.

MATERIALS AND METHODS

Site Description and Experimental Design

This study was conducted in the Living Field Laboratory (LFL) established in 1993 at the W.K Kellogg Biological Station - Long Term Ecological Research (KBS-LTER) located in Kalamazoo County, Michigan, USA. The area receives approximately 90 cm of precipitation annually, about half as snow. The site is located on a mixture of Kalamazoo and Oshtemo sandy loam soils (both Typic Hapludalfs).

The LFL was designed to investigate the effects of biodiversity (cover crops and rotational diversity) and the addition of composted dairy manure in four management systems. The focus here is on four levels of diversity, and two of the management systems: Integrated Fertilizer - IF and Integrated Compost - IC. The term "integrated" in this case refers to following recommended management practices that reduce toxicity of herbicide application (in-row banding of herbicide, and use of less toxic chemical formulations) and stringent accounting of N inputs using pre-side dress nitrate test (PSNT) and N analysis of composted dairy manure to adjust inorganic N fertilizer doses by taking into account other nitrogen sources. Synthetic fertilizer N was used in the IF systems while composted dairy manure was the primary source of N in the IC systems. Over the duration of this experiment compost with Carbon-Nitrogen (C:N) ratio ranging from 11:1 to 13:1 was applied at an annual rate of approximately 100 kg ha^{-1} of total N to all crops except soybean.

The experimental design includes every entry point in the rotation, such that each crop phase was present each year. The design is a split-split plot with four randomized complete blocks, where main plot is management system (IF and IC) and split plots for crop rotational sequence, with and without cover crops (Sanchez et al., 2004).

Individual plots were 9.1 x 20.0 m which accommodated 12 rows spaced 0.76 m apart for corn and soybean, whereas wheat was planted in 0.19 m rows. In the rotational sequence treatments we compared continuous corn with the most diverse rotation, corn-soybean-wheat.

All plots were split with and without a winter cover crop. Cover crops included red clover (*Trifolium pratense*) frost-seeded into winter wheat in March and crimson clover (*Trifolium incarnatum*) was inter-seeded in corn plots (managed with cover crops) in the initial decade of the experiment, but successful establishment of this cover crop was highly unreliable and in 2006 this treatment was replaced with seeding of a cereal rye cover crop after corn harvest.

We note that prior to 2006 the three year corn-soybean-wheat rotation sequence was a four year sequence of corn-corn-soybean-wheat. Further, in 2006 application of composted dairy manure to the IC system was suspended to allow determination of the effect of historical application.

To summarize, the treatments of interest in this study were two types of management (IF and IC), at four levels of diversity (high diversity, rotational corn-soybean-wheat with two clover cover crops and a cereal rye over time; moderately high diversity, rotational corn-soybean-wheat with no cover crops; moderate diversity, rotational corn-soybean with no cover crops, low diversity, continuous monoculture corn).

Weed management and tillage were identical in IF and IC systems, where the only major difference was historical compost use in the IC. Due to this, soil organic matter was higher in the IC than in the IF plots (Snapp et al., 2010), and following management guidelines, N fertilizer inputs were adjusted lower in IC, as indicated under crop management section.

Management

Winter cover crop split plots were maintained on the same half of each plot throughout this long-term experiment. Late March of each year red clover was frost-seeded into wheat at a rate of 20 kg seed ha^{-1}. Cereal rye was planted at a rate of 125 kg seed ha^{-1} following corn harvest within two weeks of November 1 each fall.

In the IF and IC systems, glyphosate [*N*-(phosphonomethyl) glycine] was applied at the rate of 0.5 kg ha^{-1} a.i. on the cover crop and winter fallow split plots. This was done to minimize weed biomass accumulation in the IF and IC systems. Glyphosate was applied on fallow split plots on or around 23 April.

Pre-emergence corn herbicide mixture of mesotrione {2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione}at 0.2 kg ha^{-1} a.i., *S*-metolachlor {2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl] acetamide} at 1.9 kg ha^{-1} a.i., and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) at 0.7 kg ha^{-1} a.i. were applied on all corn plots in late May. Corn insecticide {chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] was applied on continuous corn plots in both nutrient management regimes at the rate of 1.3 kg ha^{-1} a.i. at planting.

Tillage

All corn plots were chisel plowed and seed bed preparation was performed with a soil finisher/field cultivator. A row cultivator was used on all corn plots. To eliminate the effect of weed competition on plant Nitrogen availability, yield rows in both integrated fertilizer and integrated compost management treatments were hand-weeded following row cultivation each year.

Crop Management

Based on fertilizer recommendation for corn in the region, the IF system received P fertilizer in the form of triple superphosphate (0-45-0) at a rate of 50 kg ha^{-1} of P_2O_5 and K fertilizer in the form of potassium chloride (0-0-63) at a rate 84 kg ha^{-1} of K_2O, whereas the IC system had sufficient levels of P and K and did not receive fertilizer (Tri-State Fertilizer Recommendations for Corn, Soybean, Wheat, and Alfalfa).

Pioneer corn hybrid 36W66 (103 day corn) was planted in rotated and continuous corn plots at a population of 81,500 plants ha^{-1}. At 32 days after planting (DAP) plots were hand-thinned to a stand of 69,160 plant ha^{-1}.

Soil Sampling: Water Stable Aggregate Assay

Soils from each plot were sampled in November 2013 at the end of the growing season, from three depths of 0-5, 5-20 and 20 to 25 cm. The depths included the entire zone of influence associated with plant roots and cover crop residue incorporation following a sampling depth scheme of Six et al., (2000) with an addition of 20-25 cm depth following earlier surveys at the same site (Snapp et al., 2010). We used these depth increments in consistency with earlier study at the main site (Six et al., 2000). The scheme enabled us to study the zones where greater differentiation in soil aggregation attributable to agronomic treatments is expected. Visual observations of soil profiles at the site also re-affirmed our choice of the depth scheme. Five in row locations within each plot were sampled by gently hammering PVC cores into moist ground to minimize compression and slicing of soil aggregates and then pulling them with a vertical force.

Surface residues and litter were pushed aside prior to sampling so that soil C and N values and aggregate associated C reflected the mineral component only (Grandy and Robertson, 2007). Five sub samples from each plot were composited for each of the sampled plots. At the same time, three separate samples were taken for bulk density, gravimetric soil moisture and pH analyses according to KBS-LTER protocols. For water stable aggregate analysis, field moist soils were refrigerated at 4 °C at the field lab prior to being processed within 72 hours of sampling.

Soil processing was done by passing samples through an 8-mm sieve and gently breaking soil clods along natural fracture planes, and air drying for subsequent analyses. Aggregate size class distribution was determined on a triplicate of 100 g air dried composite soil sub samples for each plot by wet sieving in water at 23 °C through a series of 2000, 250 and 53 µm sieves (Grandy and Robertson, 2007). A sub-sample (100 g) of air-dried soil from each plot was then fractionated by wet sieving according to Fonte et al., (2009). These sub-samples were spread evenly onto a 2000-µm sieve and left rewetted for 5 min by spraying them with distilled water. The soil was then sieved for 2 minutes by oscillating the sieves 50 times up and down with a stroke length of 3 cm.

Large macro aggregates retained on the 2000-µm sieve mesh were backwashed into pre-weighed pans for drying. As described by Grandy and Robertson, 2007, large (>2000 µm) floating litter was removed, while soil passing through the 2000-µm sieve was transferred to a 250-µm sieve and the process was repeated to obtain the small macro aggregate fraction (250–2000 µm).

The sieving process was repeated once more using a 53-μm sieve to separate micro aggregates (53–250 μm) from the silt and clay fraction (<53 μm). All pans and soil solutions were placed in an oven at 60°C until dry. Sand content (all particles > 53 μm) was determined on all aggregate size fractions by collecting a 5g aggregate sub-sample from the collected aggregates and dispersing the sub-sample in 0.5% sodium hexametaphosphate for 24 hours on a rotary shaker at 150 rpm. Following this step, the suspension was decanted into a 53 μm sieve and sand trapped on the sieve was backwashed into pre-weighed pans, dried for 24 hours and weighed after cooling.

Computation of stability index

Mean weight diameter was computed as the summation of average aggregate size remaining on each sieve, multiplied by the percent of total sample represented by the respective aggregate class as outlined by Kemper and Rosenau (1986).

The Mean Weight Diameter (MWD) of aggregates:

$$MWD = \sum_{i=1}^{n} x_i \text{ x } w_i \qquad \textbf{(1)}$$

where;

w_i = the proportion of each aggregate class *i* to the weight of soil sample.

x_i = the mean diameter (mm) of the class (Kemper and Rosenau, 1986).

Aggregate-Associated Carbon and Course Particulate Organic Matter (cPOM)

Aggregate associated carbon concentration was determined by dry combustion methods in a CHNS analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia CA). In short, 10g sub-samples of whole soil and soil aggregates for each size fraction were pulverized in a soil mill. Organic carbon was then determined for whole soil samples and the soil aggregate size fractions. Coarse particulate organic matter (cPOM) was also determined using macro-aggregate sub-samples based on the method by Fonte et al., (2009). Sub-samples (10g) of oven-dried macro-aggregates were submerged in distilled water for 20 min to induce slaking, placed on a 250-μm sieve mesh containing 50 glass beads (4-mm diameter). Sieves were affixed to a reciprocal shaker and a slow continuous flow of water was introduced to submerge the mesh and beads in 1 cm of water and shaken for at low speed. The beads functioned to break up the macro-aggregates, while the flowing water flushed the released macro-aggregate components through the 250-μm mesh, thus avoiding further disruption of freed micro aggregates. Water and soil (<250 μm) passed to a 53-μm sieve below the reciprocal shaker to capture the released micro-aggregates. Shaking continued until water flowing onto the 53-μm sieve was clear and no aggregates remained on top of the 250-μm mesh. Material left on the 250-μm sieve was rinsed into a pan for drying. The material that remained above the 250-μm mesh was classified as coarse particulate organic matter (cPOM). Soil that passed through the 250-μm mesh onto 53-μm mesh was further processed by sieveing it for 2 min to obtain micro-aggregates within macro aggregates (>53 μm) and macro-aggregate-associated silt and clay (<53 μm). Organic C concentrations were then determined by dry combustion using a CHNS analyzer on the samples.

Statistical Analyses

Analysis of variance was performed on soil data with PROC MIXED procedure in SAS v 9.4 (SAS Institute, Cary, NC). Treatment effects on aggregate proportions, aggregate stability, SOC, POX-C were determined using ANOVA with cropping system as the fixed effect and block as a random factor. Significant differences were determined at $\alpha = 0.05$. Data were analyzed separately for each aggregate size fraction dataset. Means were separated by LSD procedure and regression analysis of POXC against the soil aggregate stability index (MWD) was performed in SAS using PROC REG procedure.

RESULTS

Soil Organic Carbon

After two decades of experimentation in the Living Field Laboratory there were differences in soil pH between integrated compost and integrated fertilizer management ($p=0.0025$, Table 1.1) for the top soil layer (0-5 cm). Overall, the IF system registered a mean pH value of 6.7 while the IC system registered mean pH 7.6 (Table 1. 2). Total Soil Organic Carbon between the two nutrient management systems were also different ($p<0.05$), but crop diversity did not influence SOC status. By 2013, SOC was higher in IC system (10.8 g kg^{-1}) representing a 21% gain compared to IF system (8.9 g kg^{-1}) for the overall depth of 0-25 cm (Table 1.2). Differences in SOC were most pronounced at upper depths: At 0-5 cm depth, mean SOC for IC was 15.0 g kg^{-1}compared to 11.1 g kg^{-1} for IF (Fig. 1.1). On the other hand, at 5-20 cm depth, mean SOC value for IC system was also higher ($p<0.05$) averaging 11.5 g kg^{-1} as compared to 9.5 g kg^{-1} for IF. At lower depths, no differences were observed between IC and IF (5.9 g kg^{-1} , 6.3 g kg^{-1} respectively, Figure 1.1).

Oxidizable Carbon

As was the case with soil organic C, the active C pool as measured by Permanganate Oxidizable Carbon (POXC) was found to have been affected by management ($p<0.05$), but not by crop diversity. For the plough layer depth of 0-25 cm, POXC was greater in IC (403 mg C kg^{-1}) than in IF (324 mg C kg^{-1}) (Table 1.2). The magnitude of differences in POXC values varied by depth being greatest in the top soil layer (0-5 cm) where the mean POXC value for IC system was 557 mg C kg^{-1} compared to 423 mg C kg^{-1} in IF system (Table 1.3). At the intermediate depth of 5-20 cm, POXC registered a mean value of 440 mg C kg^{-1} in IC system and 346 mg C kg^{-1} in IF system accordingly.

A trend similar to that observed on SOC vertical distribution was observed with the vertical distribution of POXC distribution. At lower depths (20-25 cm), differences in mean POXC values between IC and IF systems were not statistically different (213 mg C $kg^{-1}$and 204 mg C kg^{-1} respectively, Figure 1.2) among the two management systems.

Water Stable Aggregates and Aggregate Stability

Two decades after the trial was initiated in 1993, both nutrient management and crop diversity were found to have affected WSA size fractions of 2000 µm, >250 µm and > 53 µm (Table 1.4). At 0 - 5 cm the polyculture system (CSWco) under IC management registered the highest MWD (0.74). Comparatively, the mean MWD value for polyculture under IF management was 0.60. Of all treatment, the least MDW was observed in monoculture corn under IF nutrient management (0.40, Table 1.4).

Considering the entire plow depth (0-25 cm), MWD results indicated improvements of 18.5 % in IC compared to IF system (Table 1.2). Notably, there were highly significant differences in proportions of aggregate size fractions with respect to rotational diversity ($p<0.0001$) for data of the entire plow depth.

The high diversity system (CSWco) in IC had the highest mean value of large macro-aggregates (18.3 g $100g^{-1}$ soil) representing a 22% increase compared to the same system in IF. The high diversity system under IC management registered a 97% increase in comparison with the low diversity monoculture corn under IF management (0-25 cm depth, Table 1.5).

The soil aggregate stability index values were reflective of the order of complexity in the crop diversity gradient (from the low to high) as follows; C < CS < CSW < CSWco. However, small macro aggregate and micro aggregate size classes (>250 µm and > 53 µm respectively) did not necessarily reflect this order. Considering, the small macro aggregate size class (>250 µm size fraction) from soils sampled to the depth of 0-5cm, we observed higher proportions of small macro aggregates in low (monoculture), moderate (biculture) and moderately high (triculture) diversity treatments under IC management compared to corresponding diversity treatments under IF (Figure 1.5). Thus overall, IC outperformed IF with respect to proportions of WSA. Notably, the low diversity monoculture treatment in IC was associated with highest proportions of small macro aggregate. However, under IF management, this treatment had remarkably low aggregate stability (Figure 1.5).

Similar trends as noted in small macro aggregate size fraction were observed in the micro aggregate size class (>53 µm size fraction). All IC treatments showed higher proportions of micro aggregates in the monoculture, biculture and triculture systems (Figure 1.6). Likewise, no significant differences were detected among high diversity (CSWco) systems under the two nutrient management regimes (Figure 1.6).

Aggregate-Associated Soil Organic Carbon

Aggregate associated total soil organic carbon concentrations were not distributed equally among aggregate size classes. SOC levels were highest in micro aggregates (>53 µm) with a mean value of 10.3 g C kg^{-1} , seconded by large macro aggregates (>2000 µm) with a mean value of 9.6 g C kg^{-1} and least in the small macro aggregates (>250 µm) with a mean value of 8.1 g C kg^{-1} ($p<0.0001$).

In the large macro aggregate size class, (>2000 µm) diversity rather than management exerted an influence on the distribution of aggregate associated C ($p<0.05$). The highest soil C concentrations was found in large macro aggregates under high diversity treatment (10.9 g C kg^{-1}) while the lowest soil C concentration was found in large macro aggregates from low diversity treatment with a mean value of 8.3 g C kg^{-1} (Figure 1.7). The triculture and biculture systems followed with intermediate levels of 9.6 g C kg^{-1} and 9.7 g C kg^{-1} respectively that were not significantly different (Figure 1.7). On the contrary, management was found to have affected the distribution of total aggregate associated C in small macro aggregate size fraction. Higher soil C values were associated with IC system with a mean value of 9.1 g C kg^{-1} compared to 7.2 g C kg^{-1} observed under the IF system ($p<0.05$).

Surprisingly, crop diversity but not management influenced soil C distribution in the micro aggregate size class ($p<0.05$). In this size class, the highest soil C concentrations was found in the polyculture system (11.5 g C kg^{-1}) and the lowest soil C concentrations was found in the monoculture system (8.8 g C kg^{-1}) (Figure 1.8). Similar to the distribution patterns observed earlier with the large macro aggregate size class, there were no significant differences between the polyculture, triculture and biculture diversity treatments. The trend was similar to that observed with the large macro aggregate size class.

DISCUSSION

Soil Carbon Pools

Soil organic carbon status was altered by management regime over the 20 years of this study. Change in SOC is difficult to detect because of the slow pace of the processes involved in its formation and accrual. Heterogeneity of background SOC and analytical variability further compound the challenges (Kong et al., 2005) in determination of SOC changes. Of many factors affecting SOC, soil type is a key regulator of soil C status. There is a large body of literature on management having altered SOC in replicated field experiments, but most of the studies have been conducted on multiple sites with varied soil types.

Our study presented a unique opportunity to study management impacts on the same soil series and reduced inputs. Based on the findings, nutrient management had significant effects on the amount of SOC after two decades of experimentation. Soil C status in 1993 across the site at the start of the experiment was 2584 g m^{-2} (Snapp et al., 2010) and using this base line, there were significant increases in soil C status to 3672 g m^{-2} in 2013 (Table 1.6) in IF, representing a 54.4% increase with reference to the baseline. The gains in IC were 21.3 % higher in the IC system when compared to the IF system two decades from the start of the experiment (Table 1.6). In contrast to nutrient management, rotational diversity did not affect SOC changes significantly. The findings corroborate those of Snapp et al., 2010 who observed that 15 years after the start of the experiment, biodiversity had almost no effect on SOC status in the LFL among comparing organic, conventional and a combination of the two integrated management systems. However, nutrient management had marked effects on SOC status similar to our findings after 2 decades of experimentation.

The uniqueness of our study was that it compared integrated fertilizer management with integrated fertilizer management as two separate approaches, unlike in the 2010 study. In addition, we did not compare C status in organic and conventional systems as was previously done.

The effect of IC management on soil carbon accrual appears related to changes in aggregation and distribution of C in aggregate size fractions (Table 1.4). The compost management regime enhanced the proportion large macro-aggregates (>2000 µm size fraction) in the fine loamy mixed, semi active, mesic Typic Hapludalf soils of the LFL. Overall soil aggregate stability was enhanced, as evidenced by higher MWD values, most notably at the upper depth (Table 1.2). According to Denef et al., 2001, the incorporation of labile C into macro aggregates and into micro aggregates within macro aggregates decreases the rate of labile C turnover. Increase in soil C has been shown previously at this site, associated with changes in organic inputs (Sanchez et al., 2004) and changes in aggregation, particularly macro-aggregation (Grandy and Robertson, 2006). Thus compost addition in the IC system and consequential changes in aggregate formation in the IC system were likely important in building up SOC in the IC system.

Previous findings at the W.K Kellogg Biological Station have demonstrated a positive relationship between SOC accrual and increases in macro aggregates and aggregate MWD over a decade (Grandy and Robertson, 2007). However , in a cross site study, Tiemann and Grandy, 2015 found no relationship between SOC accrual and increases in aggregation and aggregate stability and attributed the lack of the relationship to the relatively short time period of the study (4 growing seasons) . Our study corroborates findings by Grandy and Robertson, 2007 and provides further evidence of a positive relationship between labile carbon accrual and increase in soil structural stability after a period of two decades (Figure 1.3).

Similar studies have failed to detect changes in some of the key soil indicators, attributing the relatively short term period (less than a decade) over which the studies were conducted as a major contributing factor (Surapur, 2014). Our study was conducted over a period of two decades and this underscores that a long period of time is required to realize appreciable changes in some of the key soil quality indicators such as water stable aggregation, when rehabilitating degraded soils.

Permanganate Oxidizable C values demonstrate that changes in POXC were also driven by management ($p<0.05$). Differences in mean POXC values between IC and IF most were pronounced in the upper portions of the soil profile (Fig. 1.2). Mean POXC values roughly reflected the same trends as SOC with respect to management and rotational diversity. Plots receiving compost had larger POXC values compared to those receiving fertilizer which corroborates findings by previous research, (Culman et al., 2013, Lucas and Weil, 2012). In another study, Culman et al. 2012 found that POXC was a more sensitive indicator of differences in management than other measured fractions in Hunter (Fertilizer, Rotation) and Watkinsville (Land Use) studies. An earlier study in the LFL indicated that both management and rotational diversity influenced POXC values, where management had a two-fold larger influence (Culman et al., 2013) than diversity.

Findings in our study indicate that POXC values and aggregate stability were positively correlated ($r^2 = 0.58$, $p<0.05$, Figure 1.3). POXC is a chemically-extractable pool of soil C that has been shown to be reflect changes in soil management practices (Culman et al., 2012).

In our study aggregate MWD and POXC showed a positive association suggesting that an increase in the labile carbon pool was associated with corresponding increase in the soil structural stability index in the LFL (Figure 1.3). Few studies have investigated the relationship between this pool of labile soil C and its implications for soil structural stability. According to Culman et al., 2012, POXC is a more sensitive indicator to changes in management practices or environmental variation and reflects a more processed, stabilized pool of labile soil C in contrast to other soil C measures such as Microbial Biomass Carbon (MBC), Particulate Organic Carbon (POC) and SOC.

Despite several reports of the usefulness of POXC as a quick and inexpensive approach to assessing changes in the labile soil C pool, little is known about the relationship of POXC with other soil quality indicators and its implications to soil health and agronomic performance. Our study aimed at addressing some of the existing knowledge gaps. We found that POXC bears a relationship with soil structural stability in the mesic Typic Hapludalf soils at the research site. To our knowledge this is the first study to explore such a relationship in a long-term integrated nutrient management system.

Water Stable Aggregates and Aggregate Stability

We found evidence that changes in soil structure were influenced by both management and rotational diversity. Changes in soil aggregation were most apparent in the top 0-5 cm compared to lower depth (Table 1.4, Table 1.5). Overall, IC system showed improved soil structural stability as evidenced by an increase in the proportion of macro-aggregates (>2000 μm) (Table 4). Cropping system diversity in the LFL was also consistently associated with increased aggregation - particularly in the case of macro-aggregates.

Our findings corroborate previous studies at the same site by Grandy and Robertson, 2007 who found a positive relationship between SOC accrual and increases in >2000 µm aggregates and aggregate MWD in a long term trial comparing four annual row crop systems, two perennial cropping systems and four native ecosystems. The improved soil structural stability associated with IC may be attributed to high levels of C inputs through compost additions in IC management of the LFL. The additions, along with stimulated microbial polysaccharides and other compounds that stabilize aggregates (Robertson et al., 1991, Angers and Mehuys, 1989) are likely factors that contribute to higher aggregate stability. Furthermore, the quality of C residue inputs from rotational diversity and quantities of those residues are likely factors that control the pace of aggregate formation across the different treatment regimes. This is shown by the markedly enhanced proportion of macro-aggregates along the crop diversity gradient Table 1.4, Table 1.5). The soil aggregate stability index values followed the order C < CS < CSW < CSWco across both nutrient management systems.

We found a marked association of rotational diversity with enhanced soil aggregate size distribution and aggregate stability, which has profound implications for ameliorating soil health in cropping systems. Enhanced C inputs from biomass produced cannot alone explain the aggregation pattern observed along the diversity gradient, as monoculture corn produces copious amounts of biomass. Quality of residues, the role of vegetative cover that is persistent over the winter (as found in the cover crop diversified systems) and root system architecture may all play a role (Grandy and Robertson 2007, Angers and Caron 1998, Kavdir and Smucker 2005). The effects of plant communities on soil structure are not well understood.

The findings in our study challenge models that predict a simple, positive relationship between organic matter C inputs and gains in aggregation and SOC. The responses observed in this study are more consistent with the hypothesis that root and associated soil biota play a more important role in aggregation.

The proportions of macro aggregates for surface soils (0-5cm) were comparatively higher compared with the results from the entire plough layer (0-25 cm) across treatments. The findings underscore the importance of considering additional factors such as depth in understanding effects of management practices on soil structural stability. Our findings demonstrate that spatial scales (vertical distribution) are also important distribution of water stable aggregates. Root dynamics, factors related to rhizospheric microbial communities affect formation processes, stabilization and patterns of macro aggregate distribution.

Results also show that the polyculture systems had similar MWD across nutrient management treatments with respect to small macro aggregates (>250 µm) and micro aggregates (>53 µm) . The findings suggest that the addition of a cover crop in the IF system renders a compensatory effect in the IF system (Figure 1.5 and Figure 1.6). Our findings contrast those of Surapur 2014, who reported no effects of winter cover crop on soil structure in a 9 year study investigating effects of cover crops across a nitrogen gradient. In addition to the relatively shorter duration of their study, conventional tillage practices in the study were thought to have likely further diminished the effects of winter cover crop biomass on soil quality.

On the contrary, previous work has demonstrated that soil aggregation can be rapidly increased by diversifying with various cover crops (Calkins and Swanson, 1998). Robertson et al., (1991) working on cover crop management of polysaccharide-mediated aggregation in orchard soils of Butte County, California demonstrated that cover crops were associated with rapid gains in the stability of soil macro aggregates. Their study investigated whether short term management of C inputs by cover crops affects polysaccharide production and polysaccharide-mediated macro aggregation in surface and subsurface soils. They found that cover crops significantly increased macro aggregate slaking resistance (soil structural stability) over clean cultivated or herbicide treatments. Similarly, Hermawan and Bomke (1997) found greater structural ability, as indicated by increases in MWD, following growth of winter cover crops on lowland soils in British Colombia. Our findings corroborate these findings and provides further evidence of the positive role of cover crops in ameliorating macro aggregate formation, stabilization and soil structural stability.

Aggregate-Associated Soil Organic Carbon

In our study micro aggregates contained high C levels compared large macro aggregates and small macro aggregates. The findings contrast those in compost-amendment study in rice systems where Sodhi et al., 2009 observed that macro aggregates had higher C compared to micro aggregates. However, our findings are in agreement with those of Jastrow et al., 1996, who reported that micro aggregates were enriched in SOC in continuous cultivated soils compared to macro aggregates.

In the LFL, SOC was highest in micro aggregates (>53 µm) with a mean value of 10.3 g C kg^{-1} , seconded by large macro aggregates (>2000 µm) with a mean value of 9.6 g C kg^{-1} and least in the small macro aggregates (>250 µm) with a mean value of 8.1 g C kg^{-1} ($p<0.0001$). Based on a conceptual model for aggregate hierarchy presented by Tisdall and Oades (1982) primary mineral particles are hypothesized to be bound together with bacterial, fungal, and plant debris into micro aggregates. These micro aggregates, in turn, are bound together into macro aggregates by transient binding agents (i.e., microbial- and plant-derived polysaccharides) and temporary binding agents (i.e., roots and fungal hyphae) and through both physical and biological perturbations soil C is believed to incorporated into aggregates where it also gets protected within the aggregates (Tisdall and Oades (1982). The process occurs in an orderly and predictable trajectory, moving from unstable macro aggregates to stable micro aggregates contained within macro aggregates (Tiemann and Grandy, 2015).

Findings from our study indicate that the amount of carbon protected in micro aggregates was greater compared to the total amount of C associated with macro aggregates. Since micro aggregate associated carbon is a protected fraction, the long term effects of integrated nutrient management systems demonstrate additional ecosystem services in terms of C sequestration. According to Grandy and Robertson, 2007 macro aggregates are highly susceptible to changes in plant community and soil disturbances. Several lines of evidence from KBS LTER trials indicate that SOC accrual occurring in macro aggregates is particularly susceptible to microbial attack and rapid turn over when the aggregates are disturbed (Tiemann and Grandy, 2015).

Interestingly, Grandy and Robertson, 2007 demonstrated that in long term grassland, a single disturbance through tillage dramatically reduced aggregation by >30% coupled with >100% increase in CO_2 fluxes. Owing to the susceptibility of macro aggregates to C losses, protection of higher quantities of soil C in micro aggregates would be more beneficial for long term carbon storage in agro ecosystems. In addition, in a long-term field experiment located at the main site neighboring the LFL, Grandy and Robertson (2007) found concentrations of C in micro aggregate size classes to vary depending on ecosystem.

Higher aggregate associated C levels were found in crops managed with moderate inputs, perennial and successional ecosystems compared to tilled, conventionally managed crops. Taken together with our findings, there is an indication that although the trajectory of aggregate formation and its contributing factors may be similar across systems, the timing and rates of these processes varies greatly under different cropping systems probably due to differences in below ground productivity (Tiemann and Grandy, 2015). Consequently, different management practices can affect C accrual across aggregate size classes in different ways necessitating closer evaluation of available management options in line with production goals.

CONCLUSION

Over two decades of experimentation, our study demonstrated that management rather than diversity had profound influence on a set of soil quality indicators such as SOC and POXC. Overall, integrated compost outperformed integrated fertilizer management in these measures. However, long term impact of rotational diversity was evident on other soil quality measures such as soil aggregate stability. Biodiverse rotations had better aggregate stability compared to corn monocultures in both management regimes. Furthermore, improvements in soil structural stability were attainable under integrated compost management in the long term relative to integrated fertilizer, crop bio-diversification with the inclusion of cover crops showed that it is an efficient means of ameliorating soil structural stability. Our findings highlight the interactions between soil physical, biological and management factors in determining the pace of trajectories in aggregate formation and stabilization and SOC accrual suggesting that although the trajectory of aggregate formation may be similar across systems, nutrient management and crop diversity gradients modulate the timing and pace of processes due to their differential effects on belowground productivity. The study provides insights on long term effects of two integrated nutrient management systems that are deployable option to conventional farming practices. The findings are important to farmers considering the use of these options and the study demonstrates some of the long term changes and the time it took to achieve the changes.

APPENDIX

Table 1.1. Management soil characteristics for 0-25 cm depth in November 2013 in the Living Field Laboratory trial at the W.K Kellogg Biological Station, Hickory Corners, MI, USA

Management	Crop Diversity	Description	pH	Bulk density (Mg m $^{-3}$)	C/N ratio
Integrated Compost	Monoculture (CC)	Continuous corn	6.67 (0.10)	1.38 (0.05)	9.7 (1.0)
	Biculture (CS)	Corn-Soy	7.09 (0.09)	1.40 (0.05)	10.0 (0.4)
	Triculture (CSW)	Corn-Soy-Wheat	7.00 (0.08)	1.36 (0.06)	9.9 (0.5)
	Polyculture (CSWco)	Corn-Soy-Wheat + cover crops	6.89 (0.07)	1.34 (0.04)	10.2 (0.4)
Integrated Fertilizer	Monoculture (CC)	Continuous corn	7.63 (0.06)	1.38 (0.03)	9.6 (0.2)
	Biculture (CS)	Corn-Soy	7.74 (0.03)	1.40 (0.05)	10.0 (0.3)
	Triculture (CSW)	Corn-Soy-Wheat	7.41 (0.15)	1.37 (0.04)	10.5 (0.5)
	Polyculture (CSWco)	Corn-Soy-Wheat + cover crops	7.77 (0.04)	1.36 (0.04)	9.7 (1.0)
ANOVA			*p value*		
Management(M)			0.0025	NS	NS
Diversity (D)			* NS	NS	NS
M x D			NS	NS	NS

Means with standard errors in parenthesis
* NS = non-significance ($\alpha = 0.05$)

Table 1.2. Effects of nutrient management on soil characteristics for 0-25 cm depth in November 2013 in the Living Field Laboratory trial at the W.K Kellogg Biological Station, Hickory Corners, MI, USA

Management	**pH**	**BD**	**SOC** g kg^{-1}	**POXC** mg C kg^{-1}	**MWD**
Integrated Compost	7.6 (0.5)	1.37 (0.02)	10.8 (0.06)	403 (25)	0.32 (0.03)
Integrated Fertilizer	6.9 (0.5)	1.38 (0.03)	8.9 (0.04)	324 (17)	0.27 (0.03)
ANOVA					
Management(M)	0.0025	NS	0.0401	0.0429	0.0149
Diversity (D)	NS*	NS	NS	NS	<0.0001
M x D	NS	NS	NS	NS	NS

Means with standard errors in parenthesis

* NS= Non-significance ($\alpha = 0.05$)

Table 1.3. Effects of nutrient management on soil characteristics for 0-5 cm depth in November 2013 in the Living Field Laboratory trial at the W.K Kellogg Biological Station, Hickory Corners, MI, USA

Management	**pH**	**BD**	**SOC** g C kg^{-1}	**POXC** mg C kg^{-1}	**MWD**
Integrated Compost	7.5 (0.1)	1.19 (0.01)	15.0 (0.07)	557 (19)	0.58 (0.03)
Integrated Fertilizer	6.7 (0.1)	1.24 (0.01)	11.1 (0.05)	423 (11)	0.48 (0.02)
ANOVA					
Management(M)	0.0060	NS	0.0108	0.0309	0.0100
Diversity (D)	NS*	0.0128	NS	NS	<0.0001
MxD	NS	NS	NS	NS	NS

Means with standard errors in parenthesis
* NS = non-significance ($\alpha = 0.05$)

Table 1.4. Management and rotational diversity effects on soil aggregation (0-5cm)

Mean water stable aggregate (WSA) size fractions, (mean ± std error) of soils sampled at 0-5 cm						
			WSA size fraction , µm			
Nutrient Management	**Crop Diversity**	**Description**	**>2000** g/ 100g soil	**>250** g/ 100g soil	**>53** g/ 100g soil	**MWD**
IC	Monoculture	Continuous Corn (CC)	19.2 ± 0.4	24.7 ± 1.8	22.4 ± 1.4	0.48 ± 0.01
	Biculture	Corn-Soy (CS)	21.1 ± 0.8	21.8 ± 1.8	20.8 ± 1.2	0.52 ± 0.02
	Triculture	Corn-Soy-Wheat (CSW)	24.4 ± 1.0	23.3 ± 1.1	22.8 ± 0.8	0.58 ± 0.02
	Polyculture	Corn-Soy-Wheat+cover (CSWco)	32.4 ± 0.9	30.1 ± 0.4	29.3 ± 1.3	0.74 ± 0.01
IF	Monoculture	Continuous Corn (CC)	16.2 ± 1.2	31.0 ± 1.3	30.1 ± 1.5	0.40 ± 0.03
	Biculture	Corn-Soy (CS)	18.2 ± 0.7	30.8 ± 1.3	29.4 ± 1.8	0.43 ± 0.02
	Triculture	Corn-Soy-Wheat (CSW)	21.7 ± 0.6	28.8 ± 0.6	26.1 ± 1.4	0.51 ± 0.01
	Polyculture	Corn-Soy-Wheat+cover (CSWco)	25.4 ± 0.7	28.3 ± 0.7	28.9 ±0.4	0.60± 0.01
ANOVA	***p Value***					
Management (M)			<0.0140	0.0125	0.0138	0.0100
Diversity (D)			<0.0001	0.0675	0.012	<0.0001
MxD			0.0225	0.0027	0.0103	0.1537

IC: Integrated Compost, IF: Integrated Fertilizer. Means ± standard errors ($\alpha = 0.05$).

Table 1.5. Management and rotational diversity effects on soil aggregation (0-25cm)

Mean water stable aggregate (WSA) size fractions, (mean ± std error) of soils sampled at 0-5 cm						
			WSA size fraction , µm			
Nutrient Management	**Crop Diversity**	**Description**	**>2000** g/ 100g soil	**>250** g/ 100g soil	**>53** g/ 100g soil	**MWD**
IC	Monoculture	Continuous Corn (CC)	11.0 ± 1.9	13.9 ± 3.7	11.6 ± 4.0	0.26 ± 0.05
	Biculture	Corn-Soy (CS)	12.4 ± 2.2	13.7 ± 3.7	10.9 ± 4.0	0.29 ± 0.05
	Triculture	Corn-Soy-Wheat (CSW)	13.6 ± 2.5	12.4 ± 3.5	10.5 ± 3.4	0.31 ± 0.06
	Polyculture	Corn-Soy-Wheat+cover (CSWco)	18.3 ± 3.2	13.8 ± 3.2	12.1 ± 3.6	0.41 ± 0.07
IF	Monoculture	Continuous Corn (CC)	9.3 ± 1.6	11.2 ± 3.0	8.5 ± 3.0	0.22 ± 0.04
	Biculture	Corn-Soy (CS)	11.2 ± 1.8	10.6 ± 2.5	8.3 ± 2.7	0.26 ± 0.04
	Triculture	Corn-Soy-Wheat (CSW)	12.6 ± 2.2	11.1 ± 2.7	9.0 ± 3.0	0.29 ± 0.05
	Polyculture	Corn-Soy-Wheat+cover (CSWco)	15.0 ± 2.5	13.6 ± 3.6	11.3 ± 3.9	0.34 ± 0.06
ANOVA	***p Value***					
Management (M)			<0.0196	0.0153	0.0087	0.0149
Diversity (D)			<0.0001	0.0089	0.0008	<0.0001
MxD			0.0352	0.0423	0.0851	0.077

IC: Integrated Compost, IF: Integrated Fertilizer. Means ± standard errors ($\alpha = 0.05$).

Table 1.6. Total C content in November 2013 for 0-25 cm depth profile, and change in soil C status since 1993 (initial soil carbon 2584 gm-2) in the Living Field Laboratory trial at the W.K Kellogg Biological Station, Hickory Corners, MI, USA

Management	**Baseline Total C content ($g\ m^{-2}$)**	**Total C content [a] ($g\ m^{-2}$)**	**Change in Total C ($g\ m^{-2}$)**
Integrated Compost	2584	3672	1088
Integrated Fertilizer	2584	3026	442

[a] Calculated based on soil bulk density of 1.38 Mg m^{-3} for both IF and IC management.

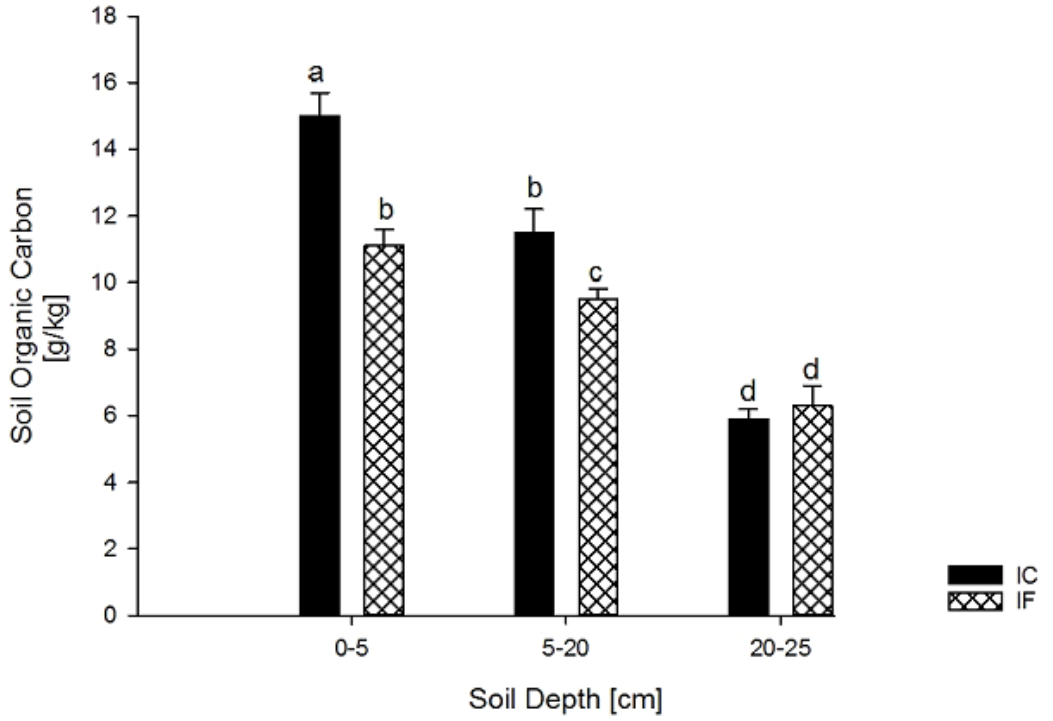

Figure 1.1. The distribution of Total Soil Organic Carbon (SOC) along the depth profile in Integrated Compost (IC) and Integrated Fertilizer management (IF) systems of the Living Field Laboratory at W.K Kellogg Biological Station, Long Term Ecological Research, Michigan in 2013. Error bars represent standard errors of the difference (SED) between means. Means indicated with the same letter are not significantly different ($p<0.05$).

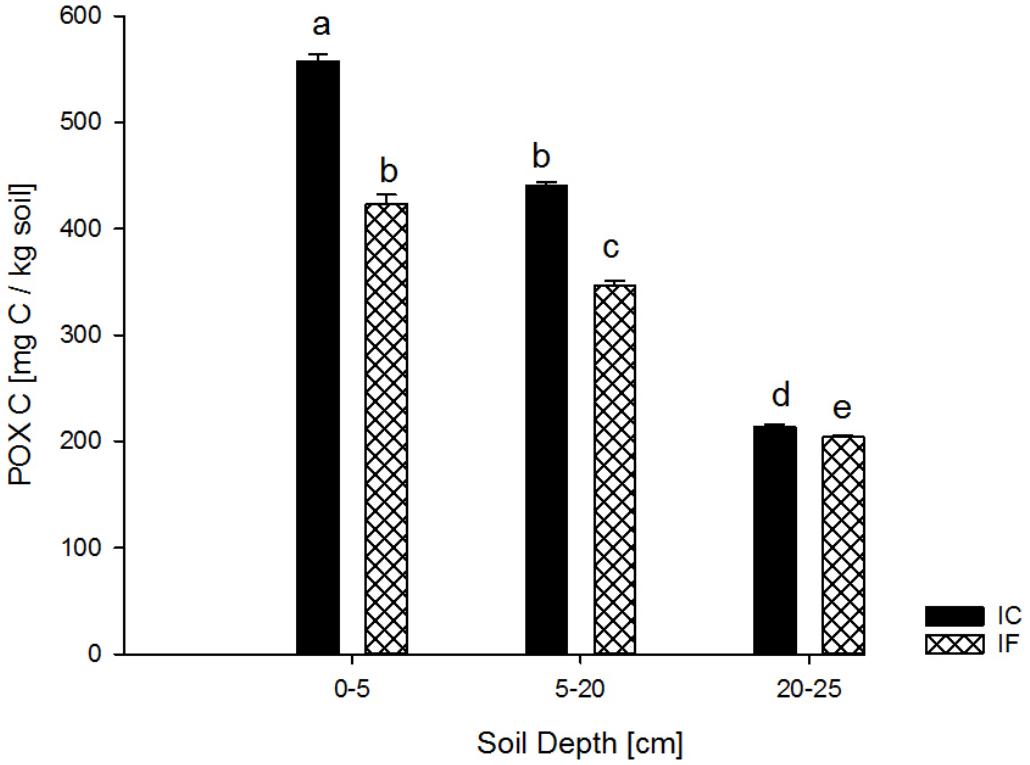

Figure 1.2. Distribution of Permanganate Oxidizable Carbon (POXC) along the depth profile in Integrated Compost (IC) and Integrated Fertilizer (IF) management systems of the Living Field Laboratory in 2013. Error bars represent standard errors of the difference (SED) between means. Means indicated with the same letter are not significantly different ($p<0.05$).

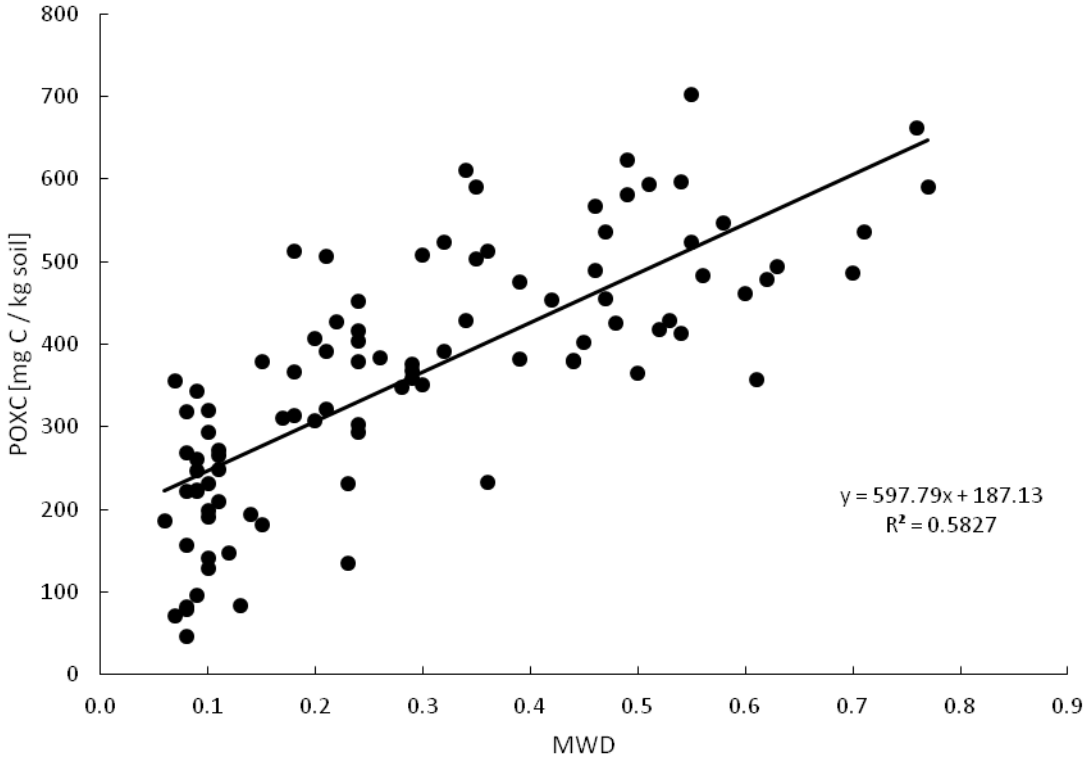

Figure 1.3. Trends in Permanganate Oxidizable Carbon (POXC) across the aggregate stability index (Mean Weight Diameter, MWD) in Integrated Nutrient management systems of the Living Field Laboratory in 2013.

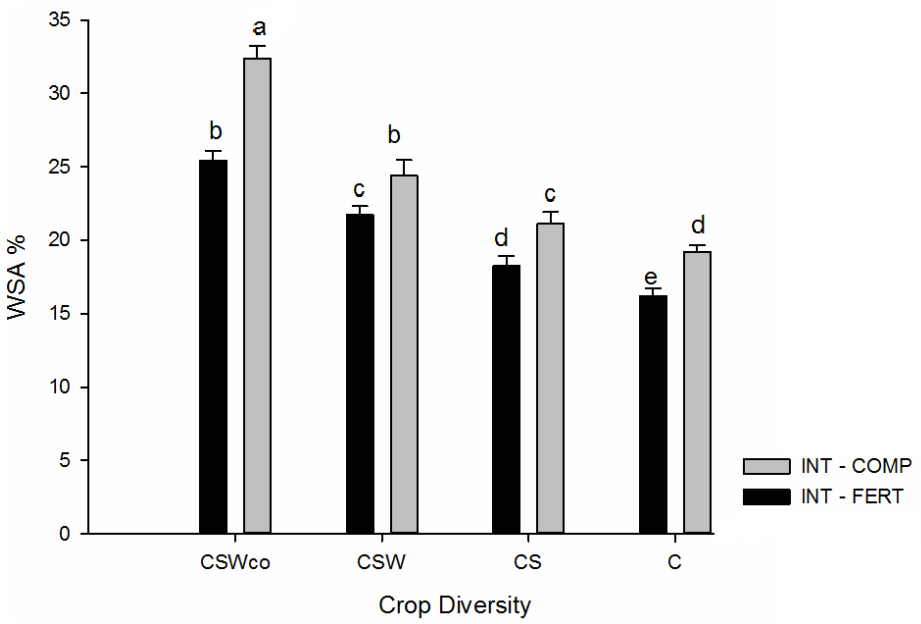

Figure 1.4. Nutrient management and rotational diversity effects on the proportion of wet sieved soil on >2000 µm size class aggregates in the Living Field Laboratory in 2013 along a depth profile of 0-5 cm. INT-COMP; Integrated Compost; INT-FERT; Integrated Fertilizer; CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors of the difference (SED) between means. Means indicated with the same letter are not significantly different ($p<0.05$).

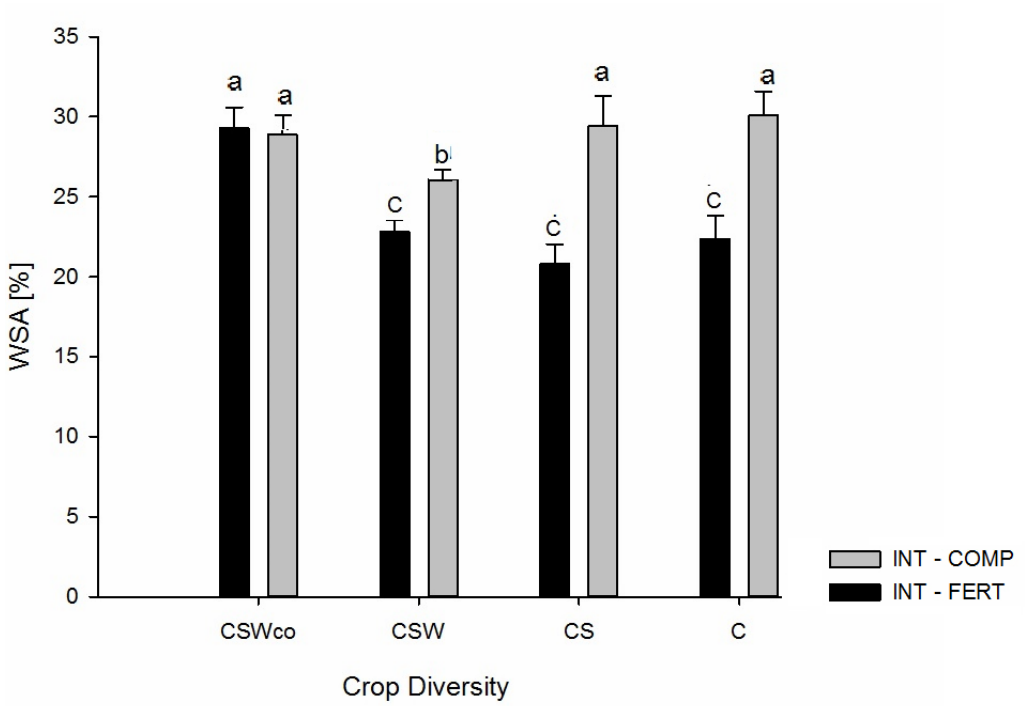

Figure 1.5. Nutrient management and rotational diversity effects on the proportion of wet sieved soil on small macro aggregates (<2000 but >250 µm in size) in the Living Field Laboratory in 2013 along a depth profile of 0-5 cm. INT-COMP; Integrated Compost; INT-FERT; Integrated Fertilizer; CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors of the difference (SED) between means. Means indicated with the same letter are not significantly different ($p<0.05$).

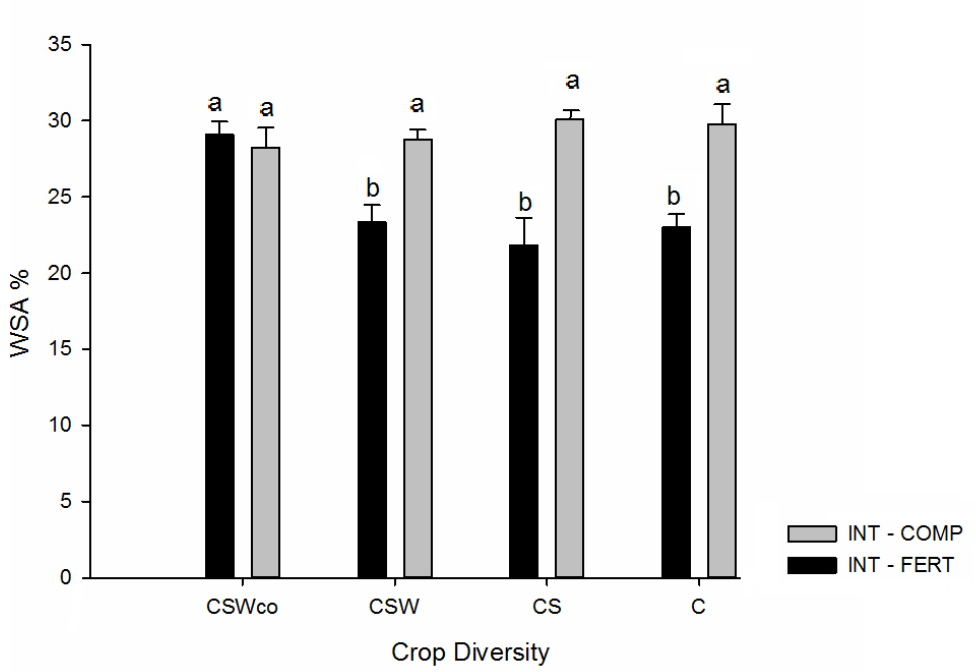

Figure 1.6. Nutrient management and rotational diversity effects on the proportion of wet sieved soil on 53µm size class aggregates in the Living Field Laboratory in 2013, sampled to the depth of 0-5 cm. INT-COMP; Integrated Compost; INT-FERT; Integrated Fertilizer; CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors of the difference (SED) between means. Means indicated with the same letter are not significantly different ($p<0.05$).

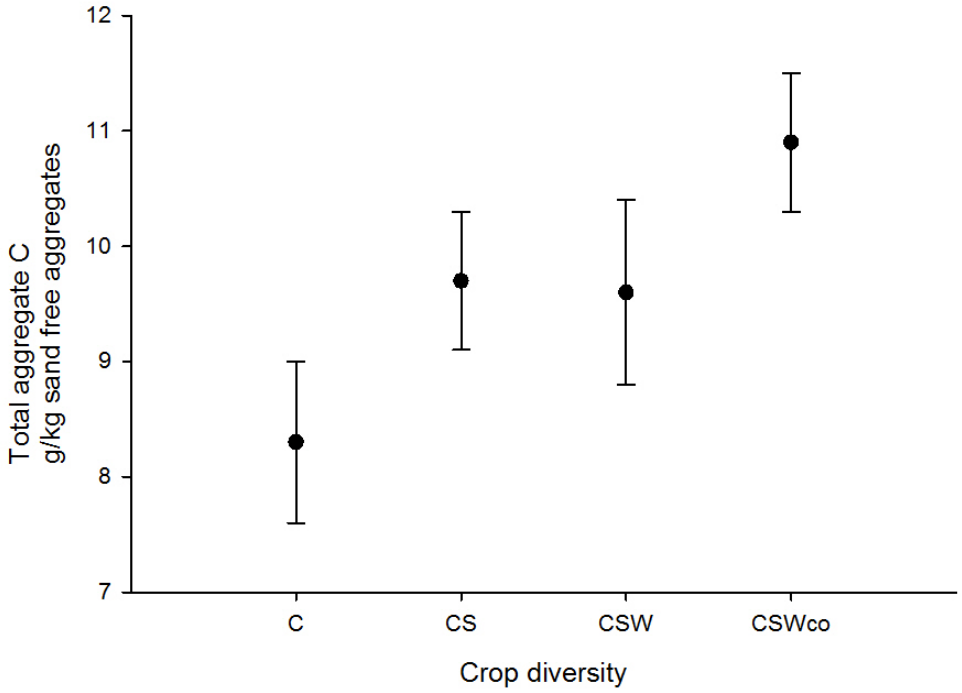

Figure 1.7. Crop diversity effects on aggregate associated total soil organic carbon concentration in large macro aggregates size class (8000-2000 μm) from LFL, KBS-LTER. C: Corn monoculture; CS: Corn Soy rotation; CSW: Corn Soy Wheat rotation; CSWco: Corn Soy Wheat rotation with cover crop. Error bars represent standard errors of the difference (SED) between means ($p<0.05$).

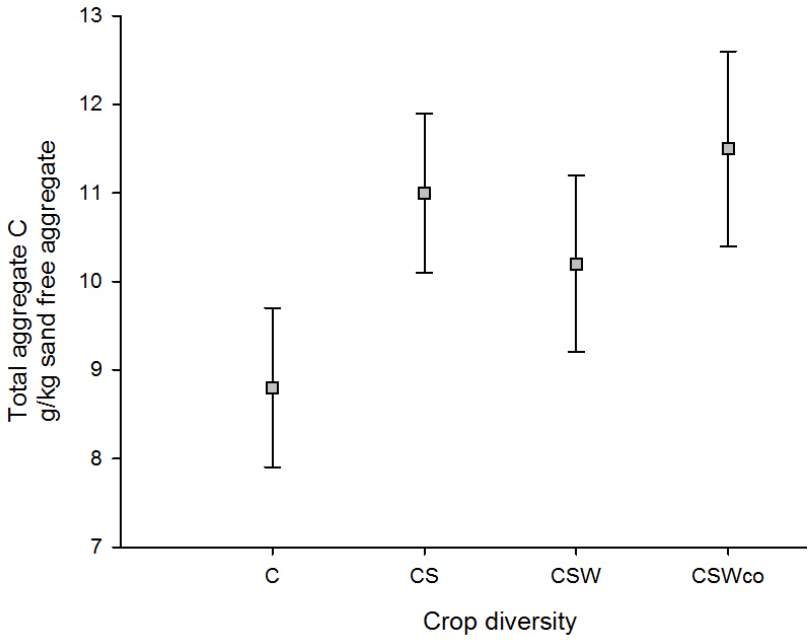

Figure 1.8. Crop diversity effects on aggregate associated total soil organic carbon concentration in micro aggregate size class (250 -53 μm) at the LFL, KBS-LTER. C: Corn monoculture; CS: Corn Soy rotation; CSW: Corn Soy Wheat rotation; CSWco: Corn Soy Wheat rotation with cover crop. Error bars represent standard errors of the difference (SED) between means ($p<0.05$).

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CHAPTER 2

INFLUENCE OF NUTRIENT MANAGEMENT AND ROTATIONAL DIVERSITY ON ARBUSCULAR MYCORRHIZAL COMMUNITY STRUCTURE AND COMPOSITION

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts that are important in agro ecosystem functioning. Conventional agriculture adversely affects AMF; yet little is known on the effects of integrated nutrient management on AMF species. We investigated AMF community responses to integrated fertilizer (IF) and integrated compost (IC) management and the role of rotational diversity on shaping AMF community structure. The Living Field Laboratory (LFL) provided a unique opportunity to test crop diversity effects using a crop diversity gradient factorially managed as IF or IC at Kellogg Biological Research Station (KBS-LTER), Southwest Michigan. The crop gradient consisted of continuous corn (C), Corn-soy rotation (CS), Corn-soy-wheat rotation (CSW), and Corn-soy-wheat with a cover crop (CSWco). Using spore morphotyping, we assessed the abundance and diversity of AMF spores among cropping systems. Proportions of AMF taxa in the systems varied with crop diversity ($p<0.05$). Nutrient management influenced soil carbon status but not AMF species community composition. A total of 9 AMF species from 4 AMF families were recovered in LFL. Shannon's diversity index was highest in polyculture systems, and lowest in monoculture corn in IC. Rotational diversity had a significant effect on spore density of AMF with higher mean spore density in polyculture system than monoculture systems. Future research should investigate if the variation in AMF community composition translates into functional differences on crop growth and productivity.

INTRODUCTION

Arbuscular mycorrhizal fungi are ubiquitous group of obligate biotrophic fungi that play key roles in the functioning and sustainability of agro ecosystems (Dai et al.,2013). AMF mutualistically associate with roots of the majority of agricultural plants and have shown the potential to increase crop productivity. AMF can increase plant nutrient uptake, reduce pathogenic infection and enhance the resistance of host plants to abiotic stresses such as drought tolerance under certain conditions (Smith and Read 1997). AMF therefore play an important ecological role in potentially influencing the plant diversity and species composition, soil aggregation, and carbon and nitrogen storage in terrestrial ecosystems (van der Heijden et al. 1998; Miller and Jastrow, 2000).

AMF share a long history of co-evolution with plants in various ecosystems resulting in their adaptation to specific natural areas (Gosling et al, 2006, Kahiluoto and Vestberg, 1998). In nature, highly mutualistic plant-AM fungal pairs are stabilized by a positive feedback loop through which mutual rewards in the form of soil nutrients are preferentially given by AM fungi to host plants in exchange for carbon (Dai et al, 2013, Kiers et al , 2011). Highly mutualistic plant-AMF pairs improve the performance of an ecosystem, in particular the efficiency of nutrient cycling, plant productivity, and the survival of AM fungi. Unfortunately, land management practices often negatively impact the stability and performance of the AM symbiosis, resulting in potential consequences on the overall productivity and sustainability of agro ecosystems (Dai et al, 2013).

Intensive agricultural practices that rely on heavy mechanization and application of synthetic fertilizers and pesticides have dramatically increased the global food supply but recent evidence suggest a number of negative environmental consequences associated with these practices. As a result there are growing concerns over the use of these practices. The concerns stem from evidence of intensive agriculture as playing a role in the contamination of groundwater, eutrophication of aquatic streams, release of greenhouse gases, loss of crop genetic diversity, loss of soil fertility increased soil compaction and poor soil structural stability (Bainard et al, 2012).

Conventional annual cropping practices have major impacts on plant cover and soil conditions, altering the conditions from their natural state (Dai et al., 2013). Consequently, conventional agricultural practices have an impact on the associated AM fungal communities in ways that may not make the practices ecologically sustainable in the long term. For instance, monoculture cropping can deprive AM fungal taxa of their host support during off season periods, and subsequently reduce AM fungal diversity (An et al., 1993, Oehl, 2003). Further, non-host crops (e.g. canola, rape seed) and fallow treatments deprive all AM fungi of an appropriate host plant (Fester and Sawers, 2011). Soil tillage and the termination of annual crops cause intense disturbance to AM fungal networks and have a negative impact on extraradical hyphal density and AM root colonization of subsequent crops (Dai et al., 2013). Of all factors, fertilization is known to strongly impact the composition, growth and function of AM fungi (Anderson et al., 1987). In general, agricultural practices have been reported to reduce the diversity and abundance of AM fungi to varying degrees depending on the intensity of crop management (Dai et al., 2013).

AM fungal communities have been shown to vary with plant community (Bever et al., 1996; Vandenkoornhuyse et al., 2002; Börstler et al., 2006; Li et al., 2010), as well as abiotic factors (Jansa et al., 2002; Oehl et al., 2003, Su and Guo, 2007). In their studies, Oel et al., 2003 and Hijri et al., 2006 demonstrated an inverse relationship between management intensity and AMF fungal richness. The concomitant shifts in AM fungal community compositions have been attributed to a number of factors which include disturbance of AMF networks, changes in soil nutrient content particularly phosphorus, altered microbial activity or changes in weed population (Jansa et al., 2003). Environment concerns associated with agricultural intensification have therefore led to the development and implementation of more sustainable agricultural practices which include the elimination of synthetic chemical inputs in organic agriculture, reduction and controlled use of synthetic chemical inputs in integrated nutrient management systems and increasing diversity by incorporating multiple crops (intercropping) or through the use of crop rotations. However, little is known on how these interventions promote or hinder AMF abundance and diversity.

The objective of this study was to evaluate the long term influence of integrated nutrient management options (Integrated fertilizer and integrated compost) and the role of rotational crop diversity on the community composition structure of AMF. We compared the species richness and relative abundance of AMF taxa along a crop diversity gradient in a 20 year study located at Kellogg Biological Station, southwest Michigan. Rotational diversity treatments were nested within two integrated nutrient management regimes.

The diversity treatments examined included continuous monoculture corn (C), Corn-soy biculture (CS), Corn-soy-wheat triculture (CSW), and a polyculture of corn-soy-wheat with a cover crop (CSWco), all with corn in the entry year. We intensively surveyed for AMF species composition and spore densities to i) determine the long term response of AMF populations to crop bio-diversification in integrated compost (IC) and integrated fertilizer (IF) management systems, and ii) examine the relationship between various soil measures to AMF diversity and composition in the fine loamy mixed, semi active, mesic Typic Hapludalf soils of the long term trial.

This study provides detailed information on the composition and diversity of indigenous AMF in a long-term field experiment that allowed us to test the hypothesis that rotational diversity influences the relative abundance and composition of AM fungal communities under two contrasting nutrient management systems. We predicted that the compost amended system would support a more diverse AMF population than the integrated fertilizer system. We also predicted that more bio diverse rotational systems would be associated with greater AM fungal diversity at the research site.

MATERIALS AND METHODS

Site Description and Experimental Design

This study was conducted on the Living Field Laboratory (LFL) established in 1993 at the W.K Kellogg Biological Station - Long Term Ecological Research (KBS-LTER) located in Kalamazoo County, Michigan, USA. The area receives approximately 90 cm of precipitation annually, about half as snow. The site is located on a mixture of Kalamazoo and Oshtemo sandy loam soils (both Typic Hapludalfs). The LFL was designed to investigate the effects of biodiversity (cover crops and rotational diversity) and the addition of composted dairy manure in four management systems (Sanchez et al., 2003). The focus in this study was on four levels of rotational diversity nested within two nutrient management systems: Integrated Fertilizer – (IF) and Integrated Compost – (IC). The term "integrated" refers to following recommended management practices that reduce toxicity of herbicide application (in-row banding of herbicide, and use of less toxic chemical formulations) and stringent accounting of N inputs using pre-sidedress nitrate test (PSNT) and N analysis of composted dairy manure to adjust inorganic N fertilizer doses by taking into account other nitrogen sources. Synthetic fertilizer N was used in the IF systems while composted dairy manure was the primary source of N in the IC systems. Over the duration of this experiment compost with carbon C:N ratios ranging from 11:1 to 13:1 was applied at an annual rate of approximately 100 kg ha^{-1} of total N to all crops except soybean. In the 2006 and 2013 seasons no compost was applied to the IC systems.

The experimental design included every entry point in the rotation, such that each crop phase was present each year. The design is a split-split plot with four randomized complete blocks, where main plot is management system (IF and IC) and split plots for crop rotational sequence, with and without cover crops (Sanchez et al., 2003). In our study, we only considered cover crops in the high diversity plots. All individual plots were 9.1 x 20.0 m which accommodated 12 rows spaced 0.76 m apart for corn and soybean, whereas wheat was planted in 0.19 m rows. In the rotational sequence treatments, we compared continuous corn with a corn-soybean rotation, corn-soybean-wheat rotation without cover crop and corn-soybean-wheat rotation with a cereal rye cover crop that replaced red clover and crimson clover seeded in the initial decade of the experiment.

We note that years before 2006, the three year corn-soybean-wheat rotation sequence was a four year sequence of corn-corn-soybean-wheat. To summarize, the treatments of interest in this study were two types of nutrient management (IF and IC), and rotational diversity at four levels of diversity (high diversity or polyculture consisting of rotational corn-soybean-wheat with cover crops; moderately high diversity or triculture consisting of rotational corn-soybean-wheat with no cover crops; moderate diversity or biculture consisting of rotational corn-soybean with no cover crops; and low diversity or monoculture comprising of continuous corn).

Weed management and tillage were identical in IF and IC systems, with the major difference of historical compost use in the IC. Due to the compost addition, soil organic matter was higher in the IC than in the IF plots (Snapp et al., 2010) and as such N fertilizer inputs were adjusted lower in IC, following nutrient management guidelines (Tri-State Fertilizer Recommendations for Corn, Soybean, Wheat, and Alfalfa, 2009).

Management

Winter cover crop split plots were maintained on the same half of each plot throughout this long-term experiment. After the start of the experiment, red clover was frost-seeded into wheat at a rate of 20 kg seed ha^{-1} in late March of each year.

Cereal rye later substituted red clover and was planted at a rate of 125 kg seed ha^{-1} following corn harvest within two weeks of November 1 each fall.

In the IF and IC systems, glyphosate [*N*-(phosphonomethyl) glycine] was applied at the rate of 0.5 kg ha^{-1} a.i. on the cover crop and winter fallow split plots. To minimize weed biomass accumulation in the IF and IC systems, glyphosate at 0.5 kg ha^{-1} a.i. was applied on the fallow split plots on or around 23 Apr., while cover crops were not sprayed with gylphosate at 0.5 kg ha^{-1} a.i. until 8 May. Pre-emergence corn herbicide mixture of mesotrione {2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione}at 0.2 kg ha^{-1} a.i., *S*-metolachlor {2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl] acetamide} at 1.9 kg ha^{-1} a.i., and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) at 0.7 kg ha^{-1} a.i. were applied on all corn plots in late May. Corn insecticide {chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] was applied on continuous corn plots at the rate of 1.3 kg ha^{-1} a.i. at planting.

All corn plots were chisel plowed and seed bed preparation was performed with a soil finisher/field cultivator. A row cultivator was used on all corn plots. To eliminate the effect of weed competition on plant N availability, yield rows were hand-weeded following row cultivation each year.

Based on fertilizer recommendation for corn in the region, the IF system received P fertilizer in the form of triple superphosphate (0-45-0) at a rate of 50 kg ha^{-1} of P_2O_5 and K fertilizer in the form of potassium chloride (0-0-63) at a rate 84 kg ha^{-1} of K_2O, whereas the IC system had sufficient levels of P and K and did not receive fertilizer (Tri-State Fertilizer Recommendations for Corn, Soybean, Wheat, and Alfalfa).

Pioneer corn hybrid 36W66 (103 day corn) was planted in rotated and continuous corn plots at a population of 81,500 plants ha^{-1}. At 32 days after planting (DAP) plots were hand-thinned to a stand of 69,160 plant ha^{-1}.

Arbuscular Mycorrhizal Fungi Spore Assay

Soil Sampling and Processing

Samples for AMF spore assay were collected from each plot at five random points within rows in on 15 October, 2013 using a core (8.25 cm diameter), to a depth of 10cm. These samples were not composited. Each sample was analyzed separately for spore diversity and abundance in the laboratory. Soil samples were kept in double polythene bags, sealed to prevent moisture loss and contamination before being transported for storage at - 4 ºC.

Isolation of arbuscular mycorrhizal fungi (AMF)

AMF spores were isolated by wet-sieving and decanting density-gradient centrifugation method as described by Schenk and Perez (1990). One hundred grams of soil sample was placed in a 2.0 L container and vigorously mixed with 1.5 L of water using a blender, to free spores from soil and roots. The suspension was left to settle for 45 min, decanted and the supernatant sieved using a series of mesh sieves stacked according to their size order (750µm, 500µm, 250µm, 100µm and 53µm at the bottom).

The sievings were transferred to 50 mL centrifuge tubes accordingly using a fine stream of water from a wash bottle. Following this step, the tubes were centrifuged at 1300 × g in a swinging bucket rotor for 3 min. The supernatant and adhering organic debris were removed carefully and the soil pellet suspended in chilled 1.7M sucrose and thereafter centrifuged at 1300 × g for 1.5 minutes. The supernatant was poured through a 53 μm mesh sieve and rinsed with tap water. Spores and sporocarps were then washed into a Petri dish and sorted into morphotypes. Representative spores were mounted on slides in polyvinyl-lactic acid-glycerol (PVLG) (Omar et al., 1979). Spores were further examined under a compound microscope and identified to the species level or attributed to a specific morphotype. Identification and classification were based on a current species descriptions and identification manual based on Schenck and Perez (1990), INVAM online references of species description (http://invam.caf.wvu.edu), University of Agriculture in Szczecin, Poland (http://www.zor.zut.edu.pl/Glomermycota/), Schüßler and Walker (2010) the website (http://www.lrz.de/~schuessler/amphylo/) accessed in July 2014.

Morphological Identification of AMF Isolates

A number of representative spores from the same morphotype were observed under a dissecting microscope at a magnification of × 50 for species identification purposes. The selected spores were put in a watch glass and their shape, size, colour, hyphal attachment, auxiliary cell, sporocarp, germination shield, and surface ornamentation observed following Morton and Redecker (2001).

Thereafter, spores were cracked open under the cover slip to allow observation of spore wall characteristics using a compound microscope. This step enabled the identification of spores to species level following classical morphological analysis (Franke-Snyder et al., 2001). AMF spores from each 100 g soil sample were counted and data expressed as mean spore density (numbers per 100 g sample).

Relative abundance of each species in each sampled plot was calculated as:

Relative abundance = $(n_i/N_j) \times 100$

where;

n_i = number of spores that belong to species *i* and N_j = total number of spores in the plot.

Mycorrhizal fungal diversity was calculated by using the Shannon index (H'), which combines two components of diversity, species richness and evenness of individuals among the species (Vestberg, 1999).

$H' = - \sum P_i \ln P_i$ and; $E = H' / H_{max}$

where;

H' = Shannon index,

P_i= proportion of the i^{th} species,

ln = natural logarithm,

E = evenness,

H_{max} = Diversity maximum when all species are equally abundant.

Spore density (SD) was expressed as the number of AMF spores per gram of soil.

Species richness (SR) was measured as a number of AMF species per sample.

Isolation frequency (IF) was computed as follows;

Isolation frequency (IF) = (the number of samples in which a given species was isolated / the total number of samples) × 100%.

Relative abundance of spores (RA) was calculated as;

RA = (number of spores in a given species / total number of spores) × 100%.

Statistical analysis

The mean of four replicate soil samples was expressed as percent relative abundance and mycorrhizal fungal diversity was calculated using the Shannon index (H'). Dominant AMF species were determined according to relative abundance (RA>5%) and isolation frequency (IF >50%) based on Li et al., 2007. Analysis of variance was performed on soil data with PROC MIXED procedure in SAS v 9.4 (SAS Institute, Cary, NC) and significant differences determined at $\alpha = 0.05$. Means were separated by LSD procedure. Regression analysis on AMF community attributes and soil properties was performed in SAS using PROC REG procedure. The relationship between AM spore density, species richness and soil parameters were finally determined by Pearson's correlation analysis.

RESULTS

Soil characteristics

Soil cores taken to the depth of 0-10 cm showed that the two management systems had similar bulk density (Table 2.1). However, nutrient management system effects were significantly different with respect to other soil properties, including pH, SOC, POXC and MWD ($p<0.05$, Table 2.2). The IC system had a more alkaline pH (7.57) compared to IF (6.76). In addition, the IC system registered higher SOC value of 13.6g C kg^{-1} soil compared to 10.6 g C kg^{-1} in IF. There were significant differences in labile soil organic carbon pool as evidenced by significantly different POXC levels, with IC associated with higher POX C value (504 mg C kg^{-1}) compared to IF (389 mg C kg^{-1}).

Relationship between AMF spore density and soil properties

Results indicated that rotational diversity rather than nutrient management system had a strong role on spore density ($p = 0.0004$, Table 2.4). We found that AMF spore density was positively correlated with soil structural stability as measured by MWD ($r^2=0.67$, $p<0.0001$, Figure 2.1). The rest of the soil properties did not show significant association with spore density (Table 2.5).

AMF spore density and crop diversity

There was no detectable influence on spore density with respect to nutrient management. However, highly significant differences were detected with respect to rotational diversity ($p = 0.0004$, Table 2.4). The spore density of AMF from high to low was CSWco > CS > CSW > C. Overall, spore density of AMF at the site was 166 ± 9 spores/100 g air dried soil.

The highest spore density level was found in polyculture systems (204 spores /100g soil). Mean spore densities for biculture and triculture systems were 166 spores /100 g soil and 163 spores /100 g soil respectively. However, the monoculture system had a mean spore density of 133 spores per 100 g soil (Table 2.4). Nine morphospecies were recovered from LFL soils and identified to species level. An additional group of unidentified spores was also found at the site and accounted for 2% of the spore population. Individual species of AMF varied in their relative abundances among different rotational diversity treatments. Largest proportion of the spores recovered from LFL soils belonged to the family Glomeraceae (5), seconded by Gigasporaceae (2). Paraglomeraceae and Claroideoglomeraceae were represented by 1 species each. Glomeraceae spores were more abundant in the polyculture, biculture and monoculture systems (Figure 2.7). However, Gigasporaceae and Claroideglomeraceae were more abundant in triculture system. In the family Glomeraceae, *S. constrictum* and *Rhizophagus intraradices* were more abundant compared to *Glomus rubiforme*, *Glomus mosseae and Glomus aggregatum* across the entire site (Figure 2.8).

AMF diversity index

Shannon-Weiner diversity index (*H´*) differed significantly among rotational diversity treatments ($p<0.001$), with significantly higher *H´* in the polyculture systems of both nutrient management systems. Low *H´* was found in the monoculture of IC. On the other hand and surprisingly, a low *H´* was also found to be associated with triculture system of IF system (Table 2.3). The Simpson diversity index indicated that rotational systems were generally more associated with an increased diversity of AMF spores compared to the monoculture system (Table 2.3).

DISCUSSION

Nutrient management and AMF community structure and composition

In this study we investigated whether ecologically sustainable agricultural systems that aim at reducing synthetic fertilizer input use and increasing crop diversity in turn promote a more abundant and diverse AM fungal community. Surprisingly, we found no evidence that nutrient management caused differences in the diversity patterns and abundance of AMF. With the exception *Claroideglomus etunicum* which was restricted to IC, the rest of AMF species were present in both nutrient management systems (IC and IF, Table 2.6). The results suggest that IC and IF systems are not so different with respect to the ability of AMF species to survive and occupy available niches. Our findings are in agreement with those of Franke-Snyder et al. 2001 who found that 15 consecutive years of corn and soy bean farming under the three management practices (conventional and two low input farming systems) did not alter fungal community dynamics. Soil properties provide some insights into nutrient management effects in the LFL. Soil organic matter was enhanced in IC (relative to IF), yet soil CN did not change. Furthermore, there were no differences in bulk density across the two nutrient management systems (Table 2.1). These and other qualitative similarities across the two management systems likely explain the lack of variability in AMF community composition across the nutrient management regimes. Furthermore, all plots in IF and IC were chisel plowed, seed bed preparation was performed with a soil finisher or field cultivator and a row cultivator was used on all corn plots. The elevated soil disturbance in all plots as a result of the tillage practice probably played a role in homogenizing AMF spore diversity across the two nutrient management systems.

Furthermore, P and K nutrition were not limiting in either system, (the IF system received P fertilizer in the form of triple superphosphate (0-45-0) at a rate of 50 kg ha^{-1} of P_2O_5 and K fertilizer in the form of potassium chloride (0-0-63) at a rate 84 kg ha^{-1} of K_2O, whereas the IC system was amended with compost and had sufficient levels of P and K levels). The application of P fertilizer in the IF system elevated P nutrient status in the IF system leading to system wide nutrient sufficiency, and less pronounced variation in mycorrhizal abundance with respect to management. Several studies have demonstrated soil P status is an important determinant of AMF spore density and abundance. Abundant P supply in the two management systems studied may have led to small benefits provided by AMF to the host plants leading to less dependency of plants on keystone AMF species. The availability of soil nutrients is an important driver in the success of host plants and feeds back to determine the success of mycorrhizal symbionts (Bever, 1997). According to Egerton-Warburton et al., 2007, the interacting effects of soil nutrients (N/P) and host plant identity determines AMF community composition. Our results provide evidence in support of these earlier findings.

Crop diversity effects on spore diversity and abundance

In contrast to nutrient management, rotational diversity markedly influenced AMF spore density in the present study (Table 2.4). After two decades of experimentation in the LFL, results showed that AMF spore density was consistently lower in monoculture compared to diverse rotations. The findings are consistent with plant community composition and structure as a major driver for AMF abundance. Two diversity measures (Shannon-Weiner and Simpson index) provided strong evidence that AM community diversity was positively influenced by plant diversity.

Seminal research was carried out on crop species diversity and AMF morphological diversity in long-term field experimentation in Minnesota (Johnson et al., 1992). The studies showed that AMF selectively proliferate in soils cropped in monoculture to corn or soybean and that mycorrhizal fungal species are individualistic in their response to cropping history and edaphic factors. Their findings indicated that the spore density of AMF species varied greatly by site. The spore density of *G. mosseae* at Lamberton plots cultivated to corn for 5 years was reported at 49.5/25 g soil while the spore density of the same species in Waseca site was 161 spores/25 g soil. In our study, the spore densities of *G. mosseae* were much lower (12 spores/100g soil in IC and 27 spores/ 100g soil in IF) compared to their study. However, both studies indicate that soil factors are important in shaping AMF species composition (Johnson et al., 1991). Further studies by Johnson showed that the relationship between spore numbers of proliferating AMF species were however negatively correlated with yield and tissue mineral concentration in the crop suggesting that proliferating VAM species in monoculture systems may be less beneficial to the crop in which they proliferate. Our study did not investigate if AMF species diversity translated into functional diversity, but tested whether crop diversity influences AMF community structure and composition. We found lower spore densities and lower species richness in corn monoculture systems compared to more diverse systems. The findings in our study provide evidence supporting shifts in AMF community structure as a consequence of rotational diversity. Schenck and Kinlock, 1980 argued in support of the notion that AMF species vary in their ability to proliferate in different crop species and although most AMF are considered to be generalists, there is some degree of specificity that exists between AMF and their host plants .

Our results provide further support on this hypothesis. In view of the extent and intensity of agriculturally managed ecosystems around the globe, it is surprising that only a handful of field studies have evaluated AMF community response to management practices in semi closed systems as alternatives to conventional management. Mathimaran et al., 2007, reported that crop rotation (maize rotated with *Crotalaria grahamiana*) affected the composition of AMF spore community although richness was not apparently influenced. In our study, several phylotypes show higher frequencies in either monoculture or polyculture systems, further supporting the previous findings. Effects of crop rotation upon AMF abundance and diversity has also been shown using *Glomus macrocarpum* (Hendrix et al., 1992) in earlier research. The principle of certain hosts being less conducive to the reproduction of particular AMF was evaluated by Hendrix and colleagues, where fescue (*Festuca arundinacea*) grown in rotation with row crops was found to be associated with decreased populations of *G. macrocarpum* below those detrimental to tobacco, whereas sorghum–sudangrass increased populations of *G. macrocarpum* (An et al., 1993; Hendrix et al., 1995) in a study by the same researchers. In another study, AMF communities were described for portions of a field after either 3 years of soybean or 2 years of fescue (An et al., 1990). Field soil from the fescue plots had 6 times as many spores as soil from continuous soybean. The MPN assays found 5 times as many propagules with fescue versus soybean. In addition, the MPN/trap culture method (An et al., 1990) yielded 13 species in the continuous soybean plots versus 16 species in the fescue. This research group found that *Glomus spp*. prevailed in rotation while *Gigaspora spp* were more numerous in continuous soybeans (An et al., 1993). The continuous soybean plots had lower species richness and diversity but higher dominance and equitability indices versus plots planted to maize, milo (*Sorghum bicolor* (L.) Moench), or fescue.

However, after a crop of soybean was grown in all plots, these differences disappeared, indicating the AMF community characterized after crop harvest reflects primarily the effects of that crop and little about previous cropping history (An et al., 1993). Due to the unique design of the LFL study which provided each phase of the rotation, we had a rare opportunity to sample from the corn phase of the rotation across all the four diversity treatments. Unlike in most studies, our study compared AMF community assemblages in rotational diversity plots with corn as the entry crop to exclude the effects emanating from crop differences as a result of rotation phase.

Ecological measures

We found that Shannon-Weiner diversity index (*H´*) differed significantly among rotational diversity treatments, with significantly higher *H´* values in the polyculture systems of both nutrient management systems. The mean Shannon-Weiner diversity index for soils in the present study was 1.94. This value is within range of values for agricultural soils in temperate agroecosystems. Franke-Snyder et al., 2001, reported a value of 1.76 for soils under conventional and low input agriculture in eastern Pennsylvannia; *H´* =0.42-1.59, Johnson et al., 1991, and *H´* = 1.81-2.22, Blaszkowski, 1995). The Simpson diversity index also indicated that rotational systems were generally more associated with an increased diversity of AMF spores compared to the monoculture system. The data indicates that there were significant differences in the evenness of the four rotational diversity treatments in the trial, reflecting the differences in the proportional abundances of spores of different species among the four communities. Significant differences were also detected in the Berger and parker indices with respect to rotational diversity (Table 2.8).

The Berger-parker indices reflect differences in the dominance of large versus small-spored species among the communities studied. The following species had their spores greater than 100 µm in diameter; *Gigaspora margarita*, *Gigaspora gigantea*, *Claroideoglomus etunicum and Glomus mosseae*. The rest of the spores had their mean diameter less than 100 µm. The biovolume of each of the AMF species detected in the study was calculated as a percentage of the total volume of spore in each pooled sample for each treatment. Over 81% of the total spore volume belonged to the Family Gigasporaceae, with *G. gigantea* taking 51 % of the total spore volume and *G. margarita* occupying 30 %. The rest of the spores from the remaining species only accounted for 19% of the total spore volume. Our findings agree with those of Franke-Synder, 2001 who reported similar findings for *G. gigantea* spores in two low input agricultural sites in eastern Pennsylvania. Gigasporaceae ranked second to Glomeraceae in spore density across the LFL. We attribute the high biovolume levels observed in the family Gigasporaceae to two factors. Firstly, spores of Gigasporaceae were found to range from two to six times larger compared to those in the family Glomeraceae, which appears to have contributed to their high biovolume levels. Secondly spores of Gigasporaceae were more evenly distributed and at moderately high levels of abundance across all the three diverse rotational systems compared to members of the family Glomeraceae. This observation is reflected in the Berger and Parker index which indicated that dominance was strong in the more diverse systems compared to monoculture systems. The combination of these two factors could explain why Gigasporaceae had the highest biovolume levels in the LFL.

Dominant AMF taxa were generally present in both nutrient management systems but not under all rotational diversity treatments. Our data supports the prediction of positive effects of crop diversity on the abundance of AMF in agroecosystems. We found that within both nutrient management systems, AMF diversity patterns were similar but the relative abundance of each species was largely influenced by crop diversity and with some species proliferating in monoculture system compared to the diverse systems.

Species richness and evenness

Our findings further indicate that the polyculture system (CSWco) had the highest species richness and spore densities of all rotational diversity treatments. According to Gao and Guo, 2010, the persistence of AMF species depends on the survival of propagules such as spores, soil mycelia and colonized root systems. The polyculture system offers spatially and temporarily heterogenous rhizospheric environments due to living roots and host residues. The phenology of different hosts in the polyculture system is varied and offers more opportunities AMF propagules across the year. The presence of cover crops in the polyculture system is important in this regard. Plant hosts with different phonologies over time and the sustained presence of cover crops in the polyculture system possibly provides a wide range of spatio–temporal niches beneficial for AMF species in the high diversity treatments. While fungal mycelia are known to survive in the soil for years (Dalpé and Aiken, 1998), spores seem to be morphologically and physiologically well preserved in over-wintering conditions. Unlike hyphae, spores also benefit from dormancy periods at low temperatures (Dalpé, 1993, Gao and Guo, 2010). Apparently, extreme conditions during winter may not be detrimental for the long term survival of spore populations and sporulating species of AMF (Gao and Guo, 2010).

Despite these mechanisms for resilience of AMF species in agro ecosystems, our data indicates that overall, two AMF species (*C. etunicum* and *G. rubi*) were absent in the monoculture treatment of the IF and one species in the monoculture treatment of the IC system (*C. etunicum*), suggesting that some AMF fungal taxa have been lost due to simplification of the monoculture system and continuous cropping. Our results concur with those of Hijri et al (2006). Dai et al., (2013) also pointed out that monoculture cropping deprives AM fungal taxa that have low compatibility with the crop plant from host support and subsequently reduces AM fungal diversity. Hijri et al., (2006) further demonstrated that fields where monoculture and other conventional practices such as intensive tillage show a lower AMF diversity. The differential effects of different hosts on AMF community structure and composition has been previously reported. Different plants have varied selective influence on extraradical AM fungal growth (Dai et al, 2013, Kiers et al., 2011), and on the structures of AMF communities in the soil (Al yahya'ei et al., 2011, Yang et al 2010).

Taxonomic diversity may not necessarily be fully reflective of the functional role of AMF in the various treatments. According to Franke-Synder et al., 2001, inter-and intraspecific variation in efficacy as symbionts has been observed in AMF populations. Individual AMF isolates can become locally adapted and even though all AMF occupy the same general niche, it is not possible to predict if functional redundancy will occur among the fungi in the community. Further research could explore the symbiotic efficacy of different AMF species to demonstrate if variability in AMF species composition or relative abundance translates into differences in capabilities to promote plant growth.

CONCLUSION

In this study, we investigated the influence of nutrient management and rotational diversity on AMF community structure and composition through a long term trial at the KBS-LTER site. We found similarities in community composition between the IF and IC nutrient management systems, with almost identical dominant AMF taxa under both management regimes. Rotational diversity emerged as the major factor behind shifts in AMF communities at the site. The increase in spore abundance and diversity associated with the polyculture system underscores additional ecosystem benefits of alternative agricultural systems or semi closed systems, whereas shifting to compost (IC vs IF) and reducing fertilizer inputs did not appreciatively alter AMF communities. Our findings highlight that more diverse rotational systems are conducive to the maintenance of a diverse AMF community and could offer an attractive option for restoration of healthy AMF communities in degraded agro ecosystems.

APPENDIX

Table 2.1. Soil characteristics of 0-10 cm depth soil profile in November 2013 in the Living Field Laboratory trial at the W.K Kellogg Biological Station, Hickory Corners, MI, USA

Management	**Crop Diversity**	**Description**	**pH**	**Bulk density ($Mg\ m^{-3}$)**	**Soil C/N ratio**
Integrated Compost	Monoculture (CC)	Continuous corn	7.55 (0.11)	1.22 (0.01)	11.36 (0.10)
	Biculture (CS)	Corn-Soy	7.69 (0.05)	1.26 (0.02)	10.62 (0.22)
	Triculture (CSW)	Corn-Soy-Wheat	7.30 (0.24)	1.20 (0.04)	10.80 (0.32)
	Polyculture (CSWco)	Corn-Soy-Wheat + 2 cover crops	7.74 (0.01)	1.22 (0.01)	10.76 (0.32)
Integrated Fertilizer	Monoculture (CC)	Continuous corn	7.63 (0.11)	1.27 (0.02)	9.98 (0.34)
	Biculture (CS)	Corn-Soy	7.74 (0.14)	1.25 (0.02)	10.46 (0.09)
	Triculture (CSW)	Corn-Soy-Wheat	7.41 (0.15)	1.24 (0.01)	10.94 (0.42)
	Polyculture (CSWco)	Corn-Soy-Wheat + 2 cover crops	7.77 (0.06)	1.23 (0.02)	10.89 (0.36)
ANOVA	*p value*				
Management(M)			0.0028	NS	NS
Diversity (D)			NS	NS	NS
M x D			NS	NS	NS

Means with standard errors in parenthesis
NS = non-significance ($\alpha = 0.05$)

Table 2.2. Spore diversity of arbuscular mycorrhizal fungi (AMF), and physicochemical properties of field soils in LFL- KBS-LTER) in 2013

	Nutrient Management	
	Integrated Compost	**Integrated Fertilizer**
Parameter		
Shannon DI	1.93 ± 0.03	1.95 ± 0.04
pH	7.57 ± 0.08 **[1]	6.76 ± 0.07
SOC (g kg^{-1})	13.60 ± 0.5*	10.60 ± 0.40
TN (g kg^{-1})	1.30 ± 0.05*	1.00 ± 0.03
CN	10.90 ± 0.2	10.60 ± 0.20
POXC (mg C kg^{-1} Soil)	504 ± 21 *	389 ± 10
MWD	0.43 ± 0.02*	0.38 ± 0.02

Shannon DI: Shannon Diversity Index; SD: Spore density; SOC: Soil Organic Carbon; TN: Total Nitrogen; CN: Carbon Nitrogen ratio; POXC: Permanganate Oxidizable Carbon; MWD: Mean Weight Diameter (Soil aggregate stability index).

Data are means ± SE

[1] All parameters were compared between Integrated compost (IC) and Integrated Fertilizer (IF) nutrient management systems by paired *t* –test; ***, $p<0.001$; ** $p<0.01$ and * $p<0.05$.

Table 2.3. α -diversity of arbuscular mycorrhizal fungi (AMF) for 0-10 cm depth in November 2013 in the Living Field Laboratory trial at the W.K Kellogg Biological Station-Long Term Ecological Research (KBS-LTER), Hickory Corners, MI, USA

Management	**Crop Diversity**	**Description**	**Shannon-Weinner**	**Simpson**
Integrated Compost	Monoculture (CC)	Continuous corn	1.76 ± 0.07	0.79 ± 0.02
	Biculture (CS)	Corn-Soy	1.91 ± 0.03	0.81 ± 0.01
	Triculture (CSW)	Corn-Soy-Wheat	2.05 ± 0.04	0.85 ± 0.01
	Polyculture (CSWco)	Corn-Soy-Wheat + cover crops	2.01 ± 0.01	0.84± 0.01
Integrated Fertilizer	Monoculture (CC)	Continuous corn	1.72 ± 0.03	0.79 ± 0.01
	Biculture (CS)	Corn-Soy	2.03 ± 0.02	0.84 ± 0.01
	Triculture (CSW)	Corn-Soy-Wheat	1.04 ± 0.08	0.85 ± 0.02
	Polyculture (CSWco)	Corn-Soy-Wheat + cover crops	2.01 ± 0.07	0.84 ± 0.02
ANOVA	*p value*			
Management(M)			NS	NS
Diversity (D)			<0.0001	0.0011
M x D			NS	NS

Data are means ± Standard Error (SE)
NS = non-significance (α = 0.05)

Table 2.4. Crop diversity effects on soil structural stability and spore density of arbuscular mycorrhizal fungi (AMF) for 0-10 cm depth in November 2013 in the LFL- KBS-LTER

Crop Diversity	**Description**	**MWD**	**Spore density**
Monoculture (CC)	Continuous corn	0.33 ± 0.01	133 ± 7
Biculture (CS)	Corn-Soy	0.38 ± 0.01	166 ± 12
Triculture (CSW)	Corn-Soy-Wheat	0.41 ± 0.10	163 ± 5
Polyculture (CSWco)	Corn-Soy-Wheat + cover crops	0.50 ± 0.02	204 ± 10
ANOVA	*p value*		
Management(M)		0.0149	NS
Diversity (D)		<0.0001	0.0004
M x D		0.0891	0.5250

Data are means ± Standard Error (SE)
NS = non-significance ($\alpha = 0.05$)

Table 2.5. Pearson-correlation matrix for edaphic variables associated with AMF spore density in LFL (KBS-LTER) in 2013

	Soil pH	BD (gcm^{-3})	SOC %	TN %	CN	POXC ($mg\ C\ kg^{-1}$ Soil)	MWD
Soil pH	-						
BD	**-0.38***	-					
SOC	**0.68*****	**-0.25**	-				
TN	**0.64*****	**-0.15**	**0.95*****	-			
CN	**0.39 ***	**-0.39***	**0.43***	**0.14**	-		
POXC	**0.74*****	**-0.23**	**0.71*****	**0.64*****	**0.32**	-	
MWD	**0.45***	**-0.25**	**0.56****	**0.55****	**0.21**	**0.56****	-
SD	**0.14**	**-0.01**	**0.21**	**0.23**	**0.05**	**0.28**	**0.67*****

BD: Bulk density; SOC: Soil Organic Carbon; TN: Total Nitrogen; CN: Carbon Nitrogen ratio; POXC: Permanganate Oxidizable Carbon; MWD: Mean Weight Diameter (Soil aggregate stability index)

An asterisk (*) signifies a difference at $p < 0.05$

(**) signifies a difference at $p < 0.01$

(***) signifies a difference at $p < 0.0001$

Table 2.6. Glomeromycota species recovered from field soils of the LFL at KBS-LTER in 2013

		Nutrient Management	
Family	**AMF species**	**Integrated Compost**	**Integrated Fertilizer**
Glomeraceae			
	Glomus constrictum	+	+
	Glomus aggregatum	+	+
	Glomus rubiforme	+	+
	Funneliformis mosseae	+	+
	Rhizophagus intraradices	+	+
Paraglomeraceae			
	Paraglomus occultum	+	+
Claroideoglomeraceae			
	Claroideglomus etunicum	+	-
Gigasporaceae			
	Gigaspora margarita	+	+
	Gigaspora gigantea	+	+

\+ denotes presence of the species
\- denotes absence of the species

Table 2.7. Diversity measures used to describe communities of arbuscular mycorrhizal fungi (AMF)

Species richness	Measured as species density (number of species / specified area)
Shannon-Weiner index of diversity ($H´$)	$H´ = - \sum P_i \ln P_i$
Evenness (E)	$E = H' / H_{max}$
Biovolume (Biovol)	$Biovol = 4/3\pi r^3$
Simpson's index (D)	$D = \sum [n_i (n_i - 1)N(N-1)]$
Modified Berger-Parker index (d)	$d = Biovol_{max} / Biovol_{total}$

Table 2.8. Diversity measurements of AMF communities in rotational diversity treatments of the Living Field Laboratory trial at the W.K Kellogg Biological Station, Hickory Corners, MI, USA

Management	**Crop Diversity**	Richness	Evenness (*E*)	Biovolume (Biovol)	MBP (*d*)
Integrated Compost	Monoculture (CC)	8	0.80 ± 0.03	2.76 ± 0.18	0.41 ± 0.03
	Biculture (CS)	9	0.83 ± 0.01	2.85 ± 0.83	0.69 ± 0.05
	Triculture (CSW)	9	0.89 ± 0.02	4.38 ± 0.68	0.67 ± 0.04
	Polyculture (CSWco)	9	0.87 ± 0.01	3.78 ± 0.41	0.51± 0.05
Integrated Fertilizer	Monoculture (CC)	7	0.83± 0.01	3.10 ± 0.61	0.48 ± 0.03
	Biculture (CS)	9	0.88 ± 0.01	2.98 ± 0.99	0.50 ± 0.03
	Triculture (CSW)	9	0.89 ± 0.04	2.25 ± 0.44	0.57 ± 0.05
	Polyculture (CSWco)	9	0.87 ± 0.03	2.89 ± 0.53	0.58 ± 0.05
ANOVA	*p value*				
Management(M)			0.2807	0.2151	0.2522
Diversity (D)			0.0166	0.8043	0.0004
M x D			0.5827	0.1622	0.0027

Data are means ± Standard Error (SE)
NS = non-significance ($\alpha = 0.05$)
MBP: Modified Berger and Parker index

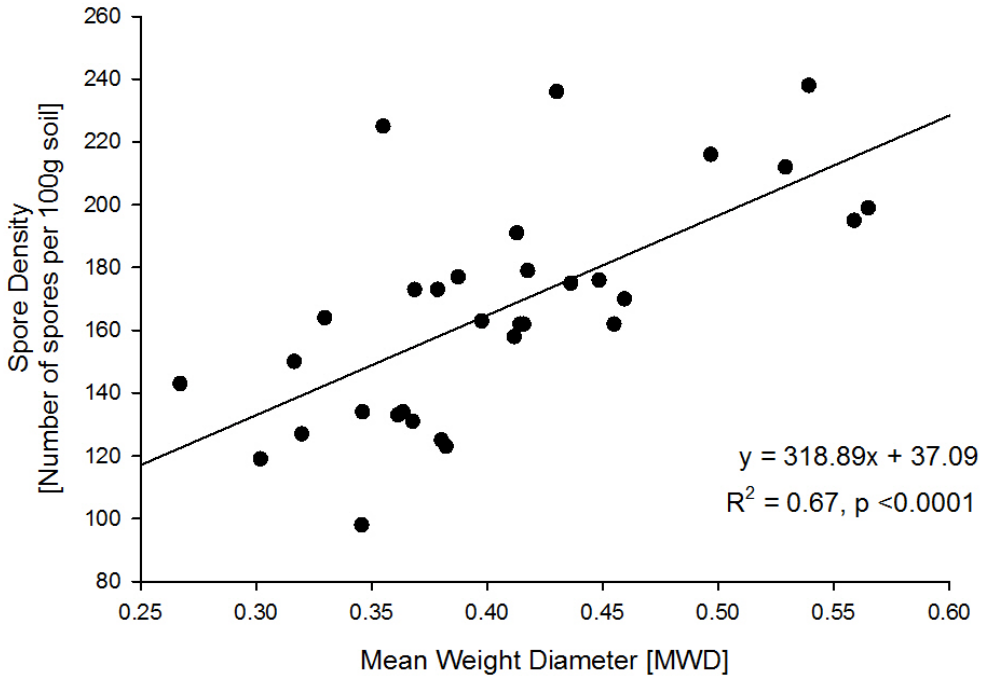

Figure 2.1. Relationship between AMF spore density and Aggregate stability measured by Mean Weight Diameter (MWD)

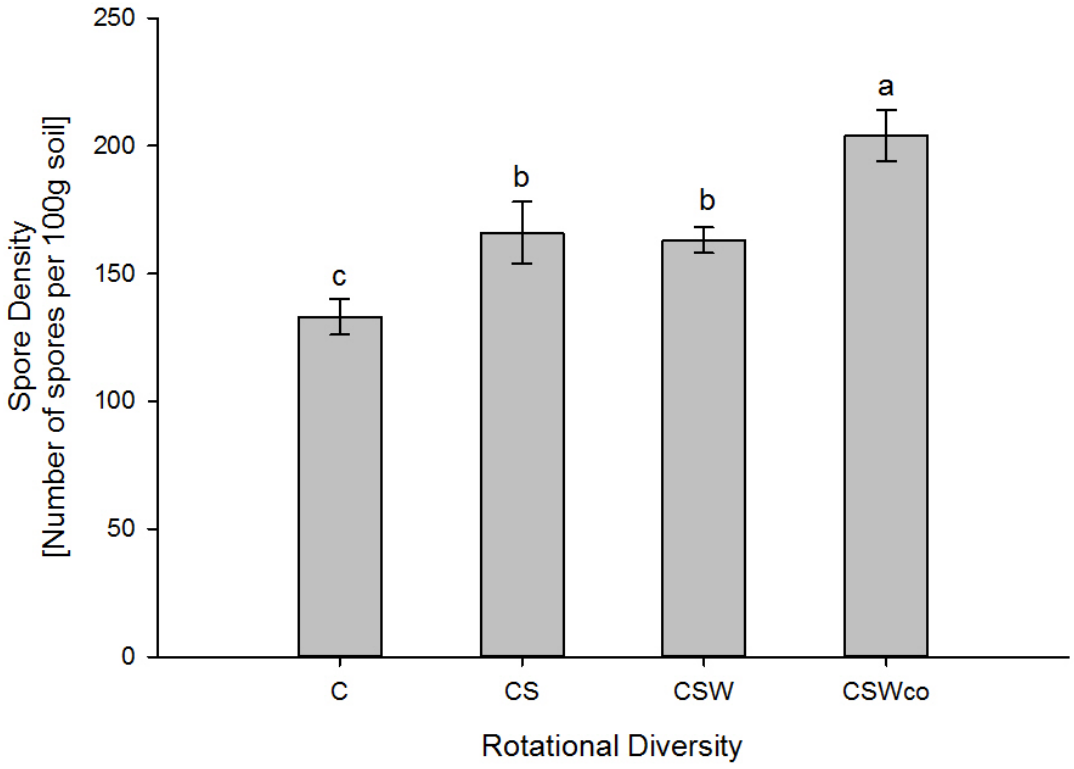

Figure 2.2. Mean AMF spore density in different rotational diversity systems. Means with the same letter are not significantly different ($p<0.05$). Rotational diversity; C: Corn monoculture; CS: Corn-Soy rotation; CSW: Corn-Soy-Wheat rotation; CSWco: Corn-Soy-Wheat rotation with cover crop.

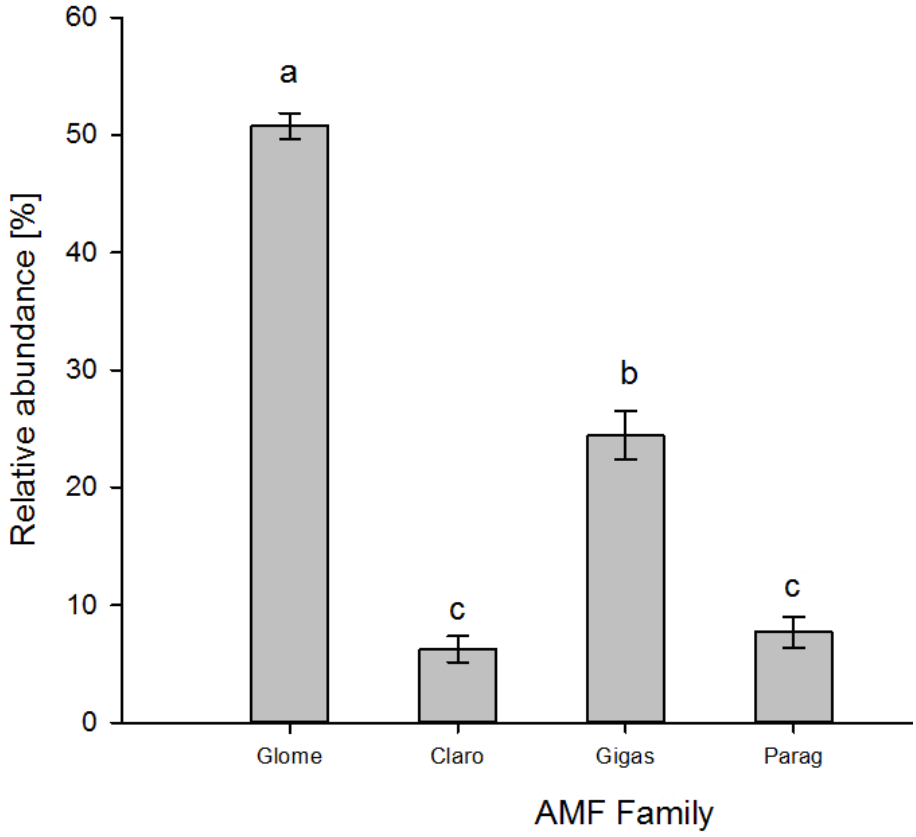

Figure 2.3. Relative abundance of AMF spores in different families from field soils of the LFL at KBS-LTER. Glome: Glomeraceae; Claro: Claroideoglomeraceae; Gigas: Gigasporaceae; Parag: Paraglomeraceae. Error bars represent standard errors (SE). Different letters denote significant differences ($p<0.05$).

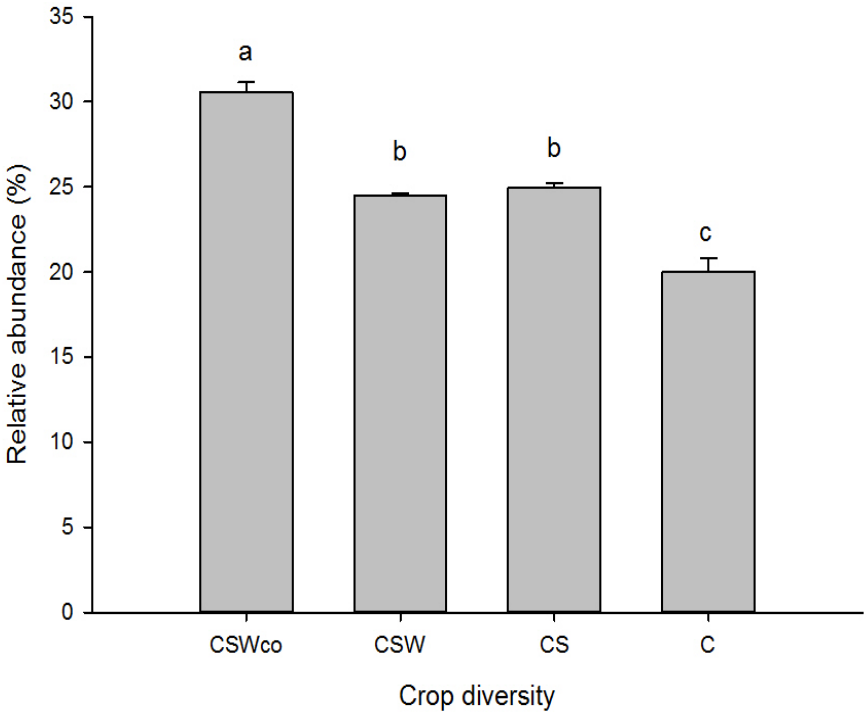

Figure 2.4. Relative abundance of AMF spores sieved from 100g of field soils in different rotational diversity systems of the LFL at KBS-LTER. CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors (SE). Different letters denote significant differences ($p<0.05$)

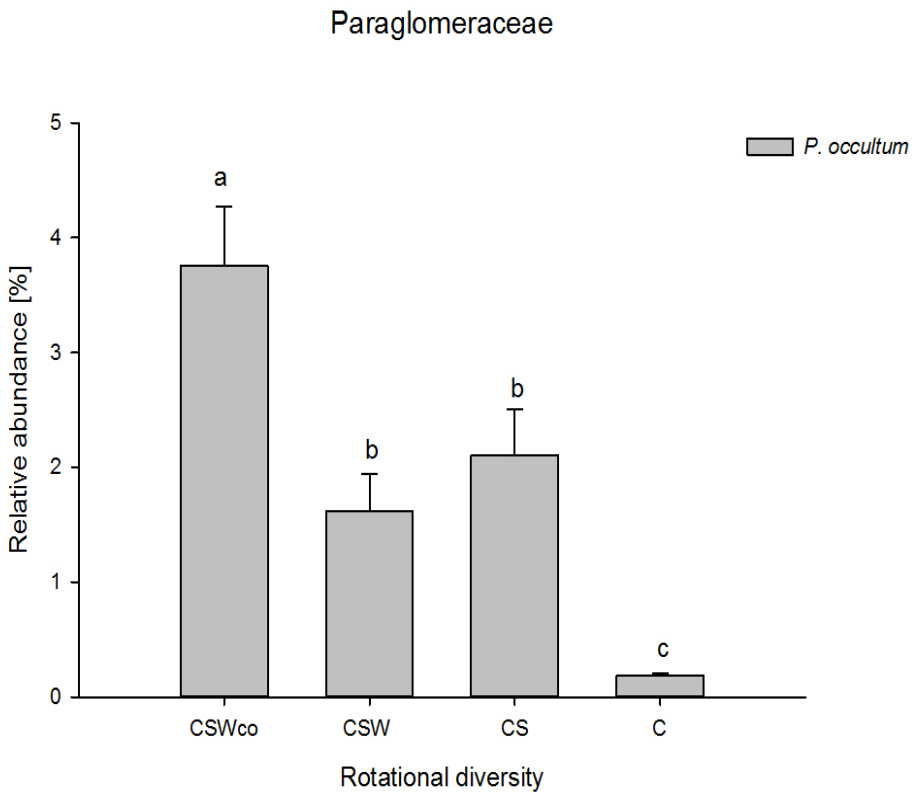

Figure 2.5. Relative abundance of AMF spores in the family Paraglomeraceae along a rotational diversity gradient of the LFL at KBS-LTER in 2013. CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors (SE). Different letters denote significant differences ($p<0.05$).

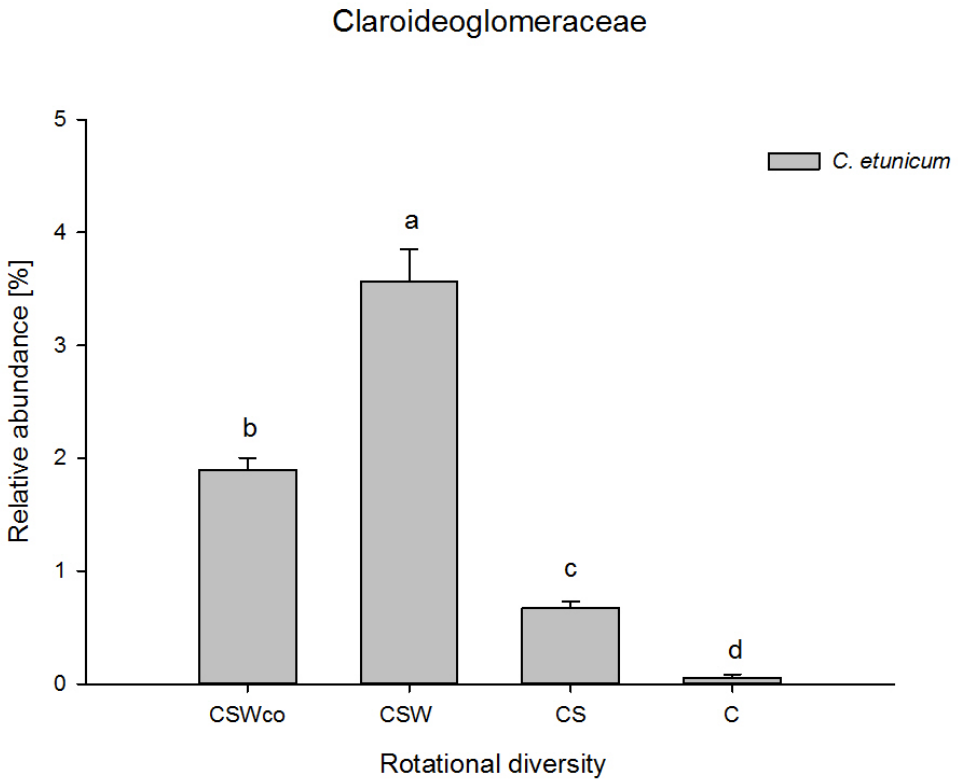

Figure 2.6. Relative abundance of AMF spores in the family Claroideoglomeraceae along a rotational diversity gradient of the LFL at KBS-LTER in 2013. CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors (SE). Different letters denote significant differences ($p<0.05$).

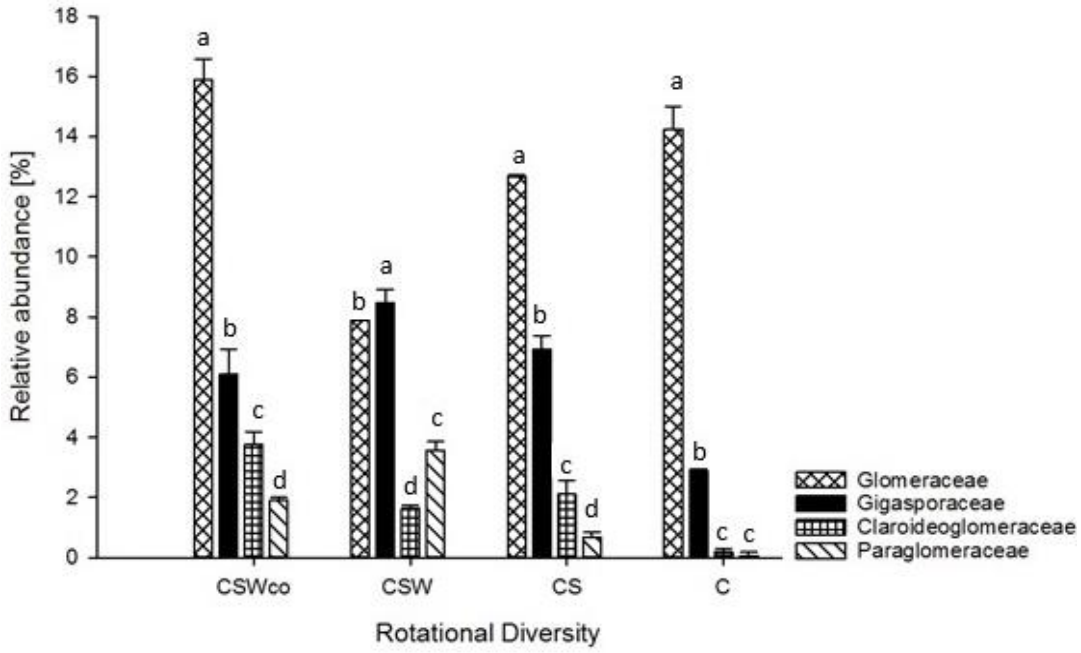

Figure 2.7. Relative abundance of AMF families sieved from 100g of field soils in different cropping systems of the LFL at KBS-LTER. CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors (SE). Different letters within a rotational diversity treatment denote significant differences ($p<0.05$).

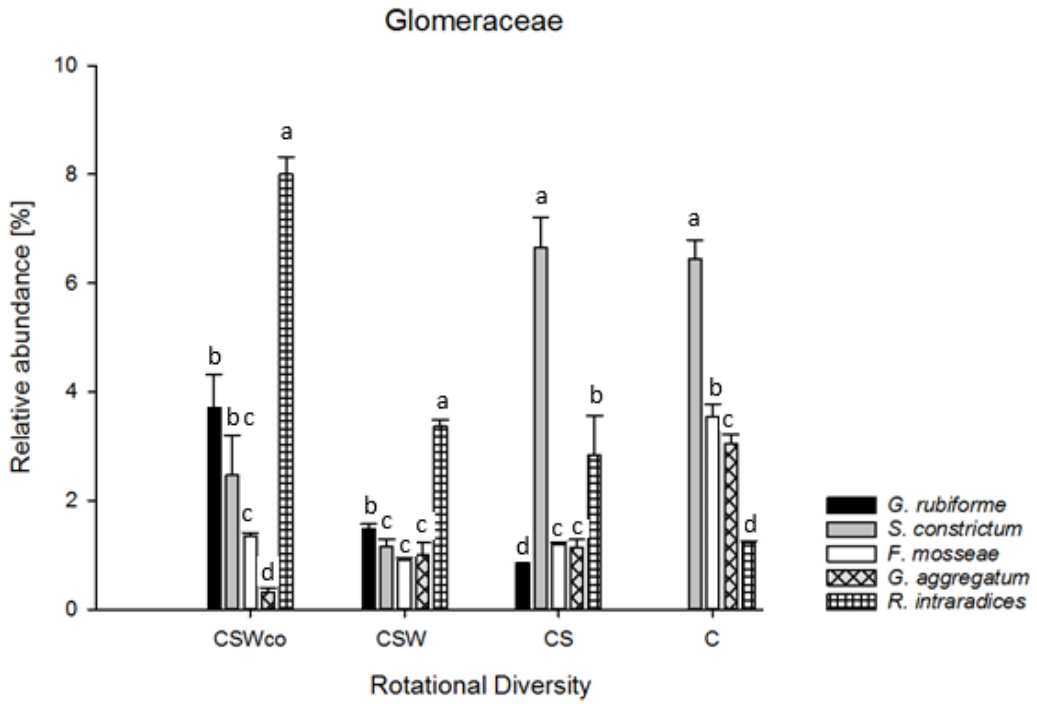

Figure 2.8. Relative abundance of AMF spores in the family Glomeraceae along a rotational diversity gradient of the LFL at KBS-LTER in 2013. CSWco: Corn Soy Wheat rotation with cover crop; CSW: Corn Soy Wheat rotation; CS: Corn Soy rotation; C: Corn monoculture. Error bars represent standard errors (SE). Different letters denote significant differences ($p<0.05$).

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CHAPTER 3

IMPACT OF LAND USE ON ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN MACHINGA DISTRICT, SOUTHERN MALAWI

ABSTRACT

The influence of land use on soil bio-resources in Sub-Saharan Africa is largely unknown. With ever increasing pressure on natural resource base, the trend of converting natural forests to croplands is only expected to increase. Natural forests in sub-Saharan Africa harbor a rich source of biota which include arbuscular mycorrhizal fungi (AMF). Yet, very little is known about the effects of conversion changes on AMF in the region. We examined communities of AMF in Miombo woodlands (natural forest) and croplands located within Malosa Forest Reserve in Machinga District, in Malawi, a country in sub-Saharan Africa. Three smallholder plots under continuous monoculture maize, three plots under maize – pigeon pea intercrop and 3 natural forest sites lying in close proximity to the croplands were included in the study. Results revealed significant variation in AMF fungal community species composition among land use types. Contrary to ecological predictions, we found no evidence of a negative effect of crop production on the taxonomic diversity of AMF. However, our study showed a significant negative influence of agricultural intensification on AMF spore abundance and AMF community structure. Further research should investigate if AMF assemblages identified vary in their functional traits and their implications to ecosystem function or agronomic performance.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are associated with a wide spectrum of plant species and are more widely distributed than other types of mycorrhizal associations (Sieverding 1991, Bedini et al. 2007, Smith and Read 2008). AMF are often important for plant nutrition and soil fertility (Smith and Read 1997, Jeffries et al. 2003) and represent a living bridge for the translocation of nutrients from the soil to the plant roots and of carbon from the plant roots to the soil (Miller and Jastrow 2000, Zhu and Miller 2003, Smith et al. 2009, Johnson et al. 2010).

AMF mutualistically associate with roots of the majority of agricultural plants and have shown the potential to increase crop productivity. AMF can increase plant nutrient uptake, reduce pathogenic infection and enhance the resistance of host plants to abiotic stresses such as drought tolerance under certain conditions (Smith and Read 1997). AMF therefore play an important ecological role in potentially influencing the plant diversity and species composition, soil aggregation, and carbon and nitrogen storage in terrestrial ecosystems (van der Heijden et al. 1998; Miller and Jastrow 2000). In nature , highly mutualistic plant-AM fungal pairs are stabilized by a positive feedback loop through which mutual rewards in the form of soil nutrients and carbon are preferentially given by AM fungi and host plants to their symbiotic partners (Dai et al, 2013, Kiers et al , 2011). Highly mutualistic plant-AM fungal pairs improve the performance of an ecosystem, in particular the efficiency of nutrient cycling, plant productivity, and the survival of AM fungi. Unfortunately, land management practices often impact the stability and performance of the AM symbiosis, resulting in negative consequences on the overall productivity and sustainability of agro ecosystems (Dai et al, 2013).

Most of the research on AMF has been done in temperate ecosystems and very little is known on the distribution of AMF in small holder farms as well as pristine ecosystems in sub Saharan Africa. Against this background, we conducted research on arbuscular mycorrhizal (AM) fungi in Miombo woodlands (natural forest) and croplands located within Malosa Forest Reserve in Machinga district, Malawi, a country in sub-Saharan Africa. The research aimed at investigating AMF species distribution across three dominant land use types lying within the same area. The work presents a starting point for testing ecological hypothesis and addressing research gaps on AMF distribution and their functional relevance in sub-Saharan Africa.

MATERIALS AND METHODS

Experimental sites

Soil samples were collected in October 2014 from agro-ecosystem and natural forest sites in Machinga District in Malawi to investigate the diversity and ecology of AMF. Studies were conducted in the Miombo forest of the Malosa Forest Reserve and cropping system sites comprising of continuous maize monoculture plots, and *Cajanus cajun* maize intercrop systems that are nested within the forest reserve. The forest reserve covers an estimated area of 86 km^2, and was gazetted as a protected area in 1924. The project sites are located between latitude 15^o 06' and 15^o 23' S and longitude 35^o 13.7' and 35^o 31' E. The forest reserve was established with the aim of protecting selected plant and large animal species, as well as the water catchment areas of Lake Malawi, the Shire River, Lake Chilwa and Lake Chiuta. The reserve was also gazetted to conserve soil, particularly in upland areas where soils are unstable and to provide sustainable commercial timber and fuel wood to communities in the country (Dudley & Kamwendo, 2004). The soil is classified as Cambic arenosols with mostly sandy top soils and low inherent soil fertility (Thierfelder et al., 2013).

Agro ecosystems

The study sites falling within agro-ecosystems lie within Malosa forest reserve, in Matandika village, Ntumbi EPA, Machinga District, Southern Malawi. The areas became deforested due to encroachment and high population pressure for growing crops and collecting wood for fuel and building material.

Maize is the main food crop grown in the area, often in a monoculture but sometimes intercropped with pigeonpea (*Cajanus cajan* L. Millsp) and cowpea (*Vigna unguiculata* L. Walp). Other crops grown in the area include groundnuts (*Arachis hypogaea* L.) and cassava (*Manihot esculenta* Crantz).

In 2006, the Department of Biological Sciences at Chancellor College, one of the constituent colleges of the University of Malawi initiated a project in the area aimed at reducing deforestation pressure in the forest reserve area by engaging rural communities in alternative livelihoods strategies. A total of 12 cropland sites and 4 natural forest sites were designated for monitoring of wild fungi in the forest reserve. In 2014, we revisited 3 of the forest sites and 6 of the cropland sites to investigate AMF spore diversity in the area. The nine sites surveyed in 2014 included the following land use classes; monoculture maize croplands, maize – pigeon pea intercrop croplands and miombo woodlands (natural forest).

Monoculture maize croplands

Monoculture maize (MM) was planted on conventional ridge and furrow system. Ridges were formed each year at 90 cm apart. Residues from the previous maize crop were placed in the furrow before forming the ridges. The ridges were then built on top of the buried residues. The in-row spacing was 30 cm. Planting was done with a hand hoe after the first planting rains. The ridges were prepared in September and October. Weed control was achieved by traditional methods with the hand hoe through re-ridging and banking, which are all meant to rebuild the ridges and achieve a weed free stand. Weeding in these sites was limited to two and sometimes three operations and stopped only when the maize reached the tasseling / silking stage.

Maize-pigeon pea intercrop croplands

Maize was planted on conventional ridge and furrow system as described above. Planting was done with first planting rains. Planting of the pigeon pea as companion crop was done in furrows after the emergence of the maize crop. No special or additional cultural practices were followed except care for pigeon pea plants during weeding. Management practices were similar as in the monoculture maize plots except for ratooning of the pigeon pea in the subsequent year. Pigeon pea stems were cut back at 30-45 cm above the ground after the onset of the first rains. Information about the field sites within agro-ecosystems was collected for each farm to document practices in previous growing seasons. This included crops grown, soil fertility management practices, period since cultivation and past land use. Maize and pigeon pea were intercropped in the recent past (less than 7 years ago). All fields had been under continuous maize cultivation since the 1990s when the land was first cleared of natural forest. Changes to maize pigeon pigeon pea intercrop on these field were introduced in 2006 during the rural livelihoods diversification project implemented by the Department of Biological Sciences, Chancellor College, Zomba, Malawi.

Host description: *Cajanus cajan*

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a drought tolerant legume of the *Fabaceae* family in the order *Fabales*. Other common names are red gram, Congo pea, Gungo pea, Gunga pea, and no-eye pea. It is the only cultivated species in the genus *Cajanus*. Initially, members of this genus were spread between two main genera; *Atylosia* and *Cajanus*. With evidence emerging from morphological, cytological and chemo-taxonomical studies, many *taxa* of *Atylosia*, found to be congeneric with *Cajanus*, were reclassified into *Cajanus* (van der Maesen, 1981). This genus now comprises 32 species from Asia, Africa and Australia (Michael, 2013).

Uses of *Cajanus cajan*

Pigeon pea is an important multi-use shrub legume for the tropics and subtropics. Widely grown for its grain that is a good source of dietary protein, pigeon pea is also grown as a vegetable for some of the poorest regions of the world (Snapp et al., 2003).

In recent years, a number of improved cultivars of pigeon pea have been released and are being disseminated to increase productivity. ICRISAT has been particularly instrumental in developing and releasing improved cultivars of pigeon pea in Malawi which include two of long duration type ('ICP 9145' and 'ICEAP 00040') and two of short duration type ('ICPL 93027' and 'ICPL 87105'). Short duration pigeon pea is largely consumed fresh as a vegetable (Simtowe et al., 2010).Apart from being an edible crop, pigeon pea (*Cajanus cajan*) is capable of fixing atmospheric nitrogen in association with *Rhizobium* bacteria. The legume biomass, when incorporated into the soil, improves soil fertility and its conditions. The grain and green leaves can be sold for cash while the dry stems make good fuel wood (Simtowe et al., 2010).

Miombo woodland characteristics

Miombo woodlands exist within a unique system called the Miombo ecoregion, characterized by several defining characteristics. Firstly, the system lies on ancient rock formations that have been geologically stable for hundreds of millions of years (Bond et al, 2010). Secondly, the system is also characterized by a long dry season over the cooler part of the year, lasting up to nine months in some areas. The third characteristic is that the system has a drainage that is generally sluggish as a result of topographical attributes and erosion that has occurred over millions of years.

Fourthly, soils of this system are ancient, developed over millions of years from the nutrient poor rocks rendering them characteristically poor in terms of nutrient content. The fourth characteristic has a bearing on the vegetation as the system has low nutrient status consequently supporting vegetation that is adapted to such a status. The final defining characteristic of this system is that it is subjected to frequent fires during the dry season (Bond et al, 2010).

The Miombo ecoregion is one of the last tropical wildernesses in the world, housing nearly 45000 endemic plants and their associated fungal flora (Byers, 2001). The ecoregion is a centre of underground trees; 86 of the 98 African species being endemic to this area representing 88% of the African underground tree species (Munishi et al, 2011). Most importantly, the system is driven by less obvious and obvious biotic factors that are inextricably linked together. These include soil fungi, termites, mega herbivores and humans that have coevolved with the rest of life forms over millions of years.

Forest fires as important drivers of the system have been an ecological factor for at least 55 000 years. Fire aids in defining the ecoregion, which contains fauna and flora with tolerance to fire (Byers, 2001). Miombo woodlands are dominated by *Brachystegia* trees which have course leaves with a lot of supporting tissue, but little nutrients. Their stems are thick and the trees form dense canopies which effectively contribute to the formation of a spectacular forest cover particularly during the rainy season. From a botanical perspective, the miombo ecoregion is quite heterogeneous and inclusive in that it consists of more than one vegetation type. Nearly six vegetation types can be found within the woodlands. However, owing to the dominance of one or more species of the *Caesalpiniodeae*, the miombo ecoregion may be referred to as the southern Caesalpinoid woodlands.

The trees that dominate this system belong to the legume sub-family *Caesalpiniodeae,* such as *Brachystegia, Julbernadia, Isoberlinia, Baikiaea, Cryptosepalum, Colophospermum* and *Burkea* (Byers, 2001). Trees shed their leaves during the dry season, and isolated Miombo trees are seen. The picture of the woodlands appears quite different at this time as often fires clear the grasses that grow underneath the trees during rainy season. By this time, the grasses have dried and fires gut the forests severely, leaving ashes and the Miombo trees standing. The trees are well adapted to this yearly phenomenon. Forest fires are often set deliberately by mice hunters, charcoal makers or villagers in the vicinity of the forests. Just before the onset of the first rains, the miombo landscape changes dramatically again. Fresh leaves appear in bright red, purple, green, cream-colored or pale greenish white. The forests appear rejuvenated in these colors for several weeks by which the leaves attain their final size and appear dark green. This period also coincides with flowering and subsequently, the first rains which usher the onset of the appearance of the ectomycorrhizal symbiotic partners.

Survey description and data collection

A survey was conducted during the dry season (October to November 2014). This survey was conducted in pre-existing land use/habitat categories lying within the same soil series but differing in land use types namely; undisturbed indigenous miombo woodlands (portion of the Machinga Forest reserve) and agro ecosystems within the reserve cultivated to maize monoculture and maize-pigeon pea intercrop. For each site plots of 30m x 30 m were used for soil sampling. Each land use type had three plots as replicates. The plots were designated Machinga miombo woodland sites 1-3 (MW1, MW2 and MW3), Matandika monoculture maize sites 1-3 (MM1, MM2, MM3) and Matandika maize- pigeon pea sites 1-3 (MP1, MP2 and MP3).

In each sampling plot for each of the three land use types, three transects were laid out to form a Z scheme, and a total of 8 points designated as sampling points along the transect. In the maize-pigeon pea sites, ICEAP 00040 was grown as the pigeon pea variety, in combination with the maize. ICEAP 00040 is a long duration variety which flowers between 140 to 180 days and matures between 190 and 240 days after sowing. The variety has a yield potential of 1500 kg/ha, considerable degree of resistance to *Fusarium* wilt and produces many spreading branches capable of growing taller than 2m (Kananji et al., 2009).

Soil analysis: Soil Sampling and Processing

Samples for AMF spore assay were collected from each plot at five points following a Z scheme to ensure random collection. Sampling was done at a single depth of 10 cm using a soil auger. In addition, three separate samples were also collected and mixed to form a composite sample which was used for physicochemical analysis. Thus a total of 8 samples were obtained from each plot. The soil samples were kept in double polythene bags, and properly sealed to prevent moisture loss and contamination before being transported to the Department of Biological Sciences research laboratory of the University of Malawi, Chancellor College in Zomba. Properties analysed included pH, soil texture, Na, K and Ca.

Isolation of arbuscular mycorrhizal fungi (AMF)

AMF spores were isolated by wet-sieving and decanting density-gradient centrifugation method as described by Schenk and Perez (1990). One hundred grams of soil sample was placed in a 2.0 L container and vigorously mixed with 1.5 L of water using a blender, to free spores from soil and roots.

The suspension was left to settle for 45 min, decanted and the supernatant sieved using a series of mesh sieves stacked according to their size order, with the largest mesh sieve at the top. The mesh sieve sizes were 750µm, 500µm, 250µm, 100µm and 53µm accordingly. The sievings were transferred to 50 mL centrifuge tubes with a fine stream of water from wash bottle and centrifuged at 1300 × g in a swinging bucket rotor for 3 min. The supernatant and adhering organic debris were removed carefully and the soil pellet suspended in 1.7M sucrose that was chilled. The suspension was then centrifuged at 1300 × g for 1.5 min. The supernatant was poured through a 53 µm mesh sieve and rinsed with tap water. Spores and sporocarps were then washed into a Petri dish and sorted into morphotypes. Representative spores were then mounted on slides in polyvinyl-lactic acid-glycerol (PVLG) (Omar et al., 1979). Spores were further examined under a compound microscope and identified to the species level or attributed to a specific morphotype. Identification and classification were based on a current species descriptions and identification manual based on Schenck and Perez (1990), INVAM online references of species description (http://invam.caf.wvu.edu), University of Agriculture in Szczecin, Poland (http://www.zor.zut.edu.pl/Glomermycota/), Schüßler and Walker (2010) and the Schüßler AMF phylogeny website (http://www.lrz.de/~schuessler/amphylo/) .

Morphological Identification of AMF Isolates

A number of selected spores from the same morphotype were observed under a dissecting microscope at a magnification of × 50 for species identification purposes. The selected spores were put in a watch glass or a small Petri dish and their shape, size, colour, hyphal attachment, auxiliary cell, sporocarp, germination shield, and surface ornamentation observed following Morton and Redecker (2001).

Thereafter , the spores were cracked open under the cover slip to allow observation of spore wall characteristics and were identified to species according to classical morphological analysis under a compound microscope (Franke-Snyder et al., 2001), and identified to species level. AMF spores from each 100 g soil sample were counted and data expressed as mean spore density (numbers per 100 g sample). Relative abundance of each species in each sampled site was calculated as:

Relative abundance = $(n_i/N_j) \times 100$

where,

n_i = number of spores that belong to species *i* and N_j = total number of spores in the site.

The mean of the replicates was expressed as percent relative abundance. Significant differences were separated by Fisher‟s LSD test at $p<0.05$ confidence level. Mycorrhizal fungal diversity was calculated by using the Shannon index (H'), which combines two components of diversity, species richness and evenness of individuals among the species (Vestberg, 1999).

$H' = - \sum P_i \ln P_i$ and; $E = H' / H_{max}$

Where, H' = Shannon index,

P_i= proportion of the i^{th} species,

ln = natural logarithm,

E = evenness,

H_{max} = Diversity maximum when all species are equally abundant.

Statistical analysis

The dominant AMF species were determined according to relative abundance (RA>5%) and isolation frequency (IF >50%) (Li et al., 2007). Analysis of variance was performed on soil data with PROC MIXED procedure in SAS v 9.4 (SAS Institute, Cary, NC). Significant differences were determined at $\alpha = 0.05$. Means were separated by LSD procedure. Regression analysis was performed in SAS using PROC REG procedure. The relationship between AM spore density, species richness and soil parameters were determined by Pearson's correlation analysis.

RESULTS

Soil physico-chemical properties

Soil chemical analyses of soil samples from the three land-use types showed that soil pH did not differ significantly among land use types (Table 3.1). However, land use type had significant effects on Soil Organic Carbon and bulk density ($p < 0.05$). SOC was significantly higher in the natural forest soils (11.9 g C kg^{-1}) compared to agricultural soils (monoculture cropland, 8.3 g C kg^{-1} and maize-pigeon pea cropland, 9.2 g C kg^{-1} respectively, Table, 3.1). In addition, bulk density was significantly lower in the natural forest soils (1.22 Mg m^{-3}) compared to agricultural soils (Table 3.1).

AMF species diversity

A total of 20 AMF species belonging to 5 families were obtained from soil samples collected from natural forest, monoculture maize crop lands and maize pigeon pea croplands (Table 3.2). Of the 20 AMF species detected, 9 belonged to the family Glomeraceae, 5 to Gigasporaceae, 4 to Acaulosporaceae, 1 to Claroideoglomeraceae and 1 to Archeosporaceae. All the five AMF families were isolated in agricultural soils but only four families were detected in natural forest soils. The family Claroideoglomeraceae, was not detected in natural forest soils (Table 3.2).

AMF diversity indices

Our results indicate that Shannon- Weiner diversity index (*H´*) differed significantly ($F_{2, 24}$ = 22.92, $p<0.0001$) among land use types with significantly higher *H´* in agricultural soils than in the natural forest soils (Table 3.3). Differences among land use types with respect to AMF diversity were also demonstrated by Simpson index (Table 3.3), reflecting community compositional and spore density differences among the communities under study. The differences in Simpson's index of dominance indicate that the pattern of spore abundance (from the most to the least dominant) were variable under the three different land use types (Table 3.3). In terms of species richness, results indicated that the three land use categories differed significantly ($p<0.05$) with respect to species richness. Natural forest soils contained the least number of AMF species (11) (Figure 3.3).

AMF spore density and land use types

AMF spore density varied significantly across land use types ($p<0.05$). Overall, the highest mean spore density for all AMF taxa recovered was found in the natural forest soils (235 spores/100 g soil) and the lowest in the maize pigeon pea croplands (188 spores/100 g soil) despite having the highest species richness (Figure 1.1). All the five AMF families were isolated in the maize pigeon pea croplands and they were represented by a total of 19 species. Of the 20 species observed across all sites, only one AMF species namely Scutellospora *pellucida* was not detected in the maize- pigeon pea crop lands. On the other hand, 17 species were isolated in the maize monoculture croplands. Results also indicated that all the five AMF families were also present in maize monoculture croplands but represented by 17 AMF species.

A total of three species represented by two species in the family Gigasporaceae and one species in the family Glomeracea were absent in maize monoculture croplands. The species were *Glomus etunicum, Scutellospora pellucida* and *Gigaspora gigantea* (Table 2.2). Surprisingly, only four AMF families namely Glomeraceae, Acaulosporaceae, Archeosporaceae and Gigasporaceae were detected in the natural forest. The family Claroideoglomeraceae was altogether undetected in the natural forest soils. A total of 11 AMF species were recovered in the natural forest soils representing a community with the lowest species richness among the three land use types investigated (Table 3.6).

Across all the three land use types, the family Glomeraceae was the most dominant family represented by 9 AMF species. Nonetheless, individual AMF species varied in their relative abundance as well as spore densities across the different land use types. For instance, *Glomus mosseae* was among the most dominant species across all the three land use types, however it was significantly higher in the maize-pigeon pea croplands compared to maize monoculture croplands and natural forest (Figure 3.5A). Within the families and across land use categories, AMF species varied in their overall spore densities and abundance (Figures 3.5 A and 3.5B). In the genus Glomus and within monoculture maize croplands, it was found that *Glomus mosseae* was the most abundant species (Figure 3.5A) and within the same genus and land use type *Glomus ambisporum* was relatively lower. Results also indicated that *Glomus etunicum* was altogether absent in monoculture maize croplands. On the other hand, *Funneliformis geosporum* ranked as the least dominant taxon in the 4 remaining genera of within the family Glomeraceae in monoculture maize croplands (Figure 3.5B).

In the maize pigeon pea crop lands, *Glomus mosseae* was likewise the greatest contributor to the overall spore abundance in the system. Five species in the genus Glomus were all present in the maize pigeon pea croplands and the least dominant species within this genus was *Glomus etunicum.*

Similar to maize monoculture crop lands, four additional genera to Glomus were each represented by one AMF species in the maize pigeon pea croplands. Once again, *Funneliformis geosporum* ranked as the least dominant taxon (Figure 3.5B) besides *Glomus etunicum* in maize pigeon pea crop lands.

Within natural forest soils, the most dominant AMF species in the genus *Glomus* was *Glomus ambisporum* (Figure 3.5A). In addition to this species, *Racocetra verrucosa* a member of the same family as *Glomus ambisporum*, also dominated the natural forest soils (Figure 3.5B). Furthermore, *R. verrucosa* was the only taxon recovered within the family but outside the genus Glomus for this land use type (Figure 3.5B). The relative abundance of *R. verrucosa* was highest in the natural forest sites.

Our results indicated that AMF species also varied in their relative abundance and spore densities across the three land use categories within the family Acaulosporaceae. *Acaulospora longula* was evenly distributed across the three land use categories, and was found to be the dominant species in both monoculture maize and maize pigeon pea crop lands (Figure 3.6). On the contrary, *Acaulospora denticulata* was associated with the highest spore density in natural forest soils. In addition, spores of *Acaulospora rehmii* were found to be less abundant in crop land soils and notably absent in natural forest soils (Figure 3.6).

Spore density patterns were also affected by land use type in the family Gigasporaceae (Figure 3.7). Results indicated that *Scutellospora cerradensis* was the most abundant within this family and restricted to the monoculture maize and maize-pigeon pea land use categories. Of the two land use categories, *S. cerradensis* was comparatively more abundant in monoculture maize croplands (Figure 3.7). *Gigaspora gigantea* was altogether absent in the monoculture maize crop lands but present in the maize pigeon pea crop land and natural forest soils. However, *Gigaspora margarita* in the same genus was abundant in forest soils and least abundant in cropland soils. Spores of *Scutellospora pellucida* were detected only in natural forest soils, albeit in small quantities (Figure 3.7).

Lastly, our results indicated that Claroideoglomeraceae and Archeosporaceae were each represented by on one AMF species. The distribution of *Claroideoglomus etunicum* was restricted to crop land soils while *Archeospora trappei*, in the family Archeosporaceae, was distributed in all the three land use categories (Table 3.2)

DISCUSSION

Effects of land use on AMF

In this study, we investigated the community composition of AMF in three distinct ecological settings and report for the first time the occurrence of AMF associated with pristine Miombo woodlands forest sites and nearby croplands in Machinga District, Southern Malawi. Our study revealed that a total of 20 AMF species were present in crop lands and the natural forest sites in the forest reserve. The number of species detected in this study falls within the range of that observed in other tropical soils (Leal et al., 2009, Jefwa et al, 2009). Our results show that (1) AM fungal community composition varied between the two cropland types and the natural forest, (2) AM fungal abundance as measured by spore density differed significantly among the three land use categories in the study (3) the maize pigeon pea cropping systems supported a richer AM fungal community compared to both monoculture maize cropping system and natural forest sites. Although this study recovered 20 AMF species using morphological characterization of spores in soil samples, we take cognizance of the fact that the number may not represent the total alpha diversity of AMF occurring in the systems as other factors such seasonality and the challenge to capture non-sporulating species can influence species richness and abundance. Nevertheless, AMF species richness in our study is higher than that reported by Jefwa (2009) and nearly of equal magnitude as reported by Leal et al., (2009) in other tropical sites.

AMF species community composition

The impact of land use on community structure and composition of AM fungi in miombo woodlands and nested agroecosystems is largely unknown. We found surprising results indicating that AM fungal diversity was lower in the natural forest compared to croplands nested within the forest reserve. Many studies have reported negative effects of agriculture on AM fungal richness. It is well documented that soil tillage which is a common practice in crop production has a negative impact on AM hyphal network biomass and infectivity (Dai et al, 2013). But our findings point out that the influence of tillage (done by hand hoes in this case) was likely minimal. Other factors such as intensive use of agricultural inputs are also known to negatively affect AM fungal richness. Addition of nitrogen fertilizers to the soil decrease soil pH and can affect AM fungal richness in croplands, but smallholder croplands in the region are known to be nutrient deficient, with little or no information on the nutrient status of the farms such that despite the addition of nitrogen fertilizers, the quantities may not be sufficient enough to make the appreciable difference. Our results suggest that all the three land use types examined in the study had similar pH implying that nitrogen fertilizer additions did not result in major changes in the soil chemical properties. Furthermore, the results indicate that pH was not a major driver of the AM fungal diversity in the three land use types.

In our study, five AMF families were recovered from the field soils collected from croplands while four families were recovered from soils sampled from natural forest sites. Our results indicated that of the five families detected in the study, the family Clairoideoglomeraceae was absent in the forest soils.

Measures of species diversity demonstrated that AMF species diversity was low in natural forest soils compared to cropland soils. The findings concur with those of Leal et al.,2009 who observed that tropical crop lands harbored more AMF genera (or families) compared to Forest ecosystems in a study conducted in the Amazon. In their study, croplands were shown to have harboured six AMF genera (*Acaulospora, Paraglomus, Entrospora, Glomus, Scutellospora* and *Archeospora*) while forest land use systems harboured only three genera (*Acaulospora, Glomus* and *Gigaspora*). Our findings also corroborate those of Moora et al., 2014 who found that AMF richness was relatively low in forest ecosystems compared to structurally open ecosystems (arable lands) and permanent grasslands. In another study, Dai et al., 2013, demonstrated that croplands in the Atlantic maritime hosted richer AM fungal communities compared to semi-natural areas, disapproving the hypothesis of a negative effect of agriculture on AM fungal community assemblages. The findings suggest that semi-natural and natural areas may not necessarily act as reservoirs harboring diverse AMF for the conservation of AM fungal diversity.

Several factors may be attributed to the low AMF richness in natural areas such as forest ecosystems. In their study, Moora et al., 2014 attributed the observed patterns of low AM fungal richness associated with forest ecosystems to differences in the pH of the top soil, with the pH in the forest ecosystems being lower than in the open systems, and acidity being negatively correlated to AMF richness. In our study, we did not find significant differences in the pH among the three land use types at the depth of 0-10 cm. The findings suggest that pH alone may not be a stronger factor in structuring of AM fungal communities in the different land use types under investigation.

Other factors may play more pivotal role in the structuring of AM fungal communities. In our study, forest ecosystems were predominated by ectomycorrhizal miombo tree species. Previous studies have shown that ectomycorrhizal trees can suppress AM fungi in soils (Tyndall, 2005) as well as AMF root colonization (Becklin et al., 2012). Our findings support the notion that natural woodlands dominated by ectomycorrhizal tree species such as those in the tropical Miombo forest of southern Africa could be associated with reduced AMF diversity. In their study, Koorem et al., 2011 found that spruce forest had low AMF diversity and abundance and attributed their finding to the inhibiting effects of spruce litter on the emergency and establishment of seedlings and their AMF symbionts. According to Moora et al., 2014, lower AM fungal taxon richness in forested habitats compared to arable lands may be a result of complex influence of ectomycorrhizal plants and their associated ectomycorrhizal fungal partners through suppression of AM plant species in the understory. In addition, the acidification of top soil due to leaf litter may further negatively influence AM fungal richness, leading to lower AM fungal richness in forest areas.

Our study also indicated that monoculture maize croplands had comparatively lower AM fungal richness compared to maize-pigeon pea intercrop croplands. The findings corroborate those of Bainard et al., 2012 who demonstrated significant compositional differences between conventional monocropping of corn and tree-based intercropping systems and indicated that conventional monocropping systems had lower phylotype richness compared to tree based intercropping systems. In our study, the observed pattern may be linked to the greater plant diversity in the maize-pigeon sites compared to the monoculture maize sites.

Previous studies have shown that host plant community composition can influence the diversity and composition of AM fungal communities especially in soils with a small range of soil chemical factors (Helgason et al., 2007; Dumbrell et al., 2010).

Our findings also indicate that AMF spore density differed significantly among the three land use types studied with the highest overall spore density associated with natural forest soils and the lowest in the maize pigeon pea crop lands. Results revealed that maize pigeon pea croplands haboured many rare species. Only *Archeospora trappei* , the sole representative in the family Archeosporaceae was dominant in maize pigeon pea croplands. Although this member was associated with the highest spore density, overall the mean spore density for this land use category was the lowest. On the other hand, monoculture maize croplands and natural forest sites haboured several dominating species which made significant contributed to the overall spore density. Glomeraceae and Archeosporaceae were dominant families in the forest sites while Claroideoglomeraceae and Archeosporaceae were dominant in maize monoculture croplands (Figure 3.2). The observation suggest that mycorrhizal communities occurring in the three different landuse types are comprised of AMF species with distinct r and k strategies (Souza and Declerek, 2003; Sturmer, 1998). High sporulation of AMF species in the forest sites might reflect an r strategy of some species that allocate most of the carbon to sporulation while a k strategy might be represented by species that are not prolific sporulators and allocate resources to vegetative growth (Leal et al 2009). The natural forest sites are typically miombo woodlands characterized by a long dry season, soils that are poor in nutrients and frequent fires during the dry season (Bond et al, 2010).

Considering these characteristics, environmental fitness may have developed as an adaptive strategy leading to greater investment in sporulation than vegetative growth. Other factors could also be responsible for the observed patterns. Croplands likely harbor populations of different predators and parasites leading to AMF spore predation and hyperparasitism which might result in loss of spore viability and unavailability by the time of sampling. In addition, natural forest as represented the miombo woodlands in the study are dominated by various perennial plants and are probably more stable than croplands regarding the presence of AMF hosts across seasons unlike in the croplands, which could act as selection pressure on fungal assemblages that keep sporulating across seasons. In the same vein, Sasvari et al., 2001 studying the community structure of AM fungi in roots of maize grown in a 50 year monoculture found out that spore density increased through the growing season and peaked during the reproductive stages of maize and subsequently declined during at the end of the growing season. In our study, sampling was done at the end of the maize growing season. The observed increase in spore density in the natural forest sites may be a function of the different AM fungal community in the forest sites compared to the two croplands. Many studies have reported evidence of contrasting seasonal sporulation dynamics among AMF species which may account for the observed trends in natural forest sites and the croplands (Bainard et al., 2012).

Our results demonstrated that the family Glomeraceae was the most dominant across all the three land use types. Within this family the genus *Glomus mosseae* was the most abundant in terms of spore density.

The findings concur with those by previous researchers who found Glomus to be the most abundant and widespread AMF phylotype in nature that exhibits high local abundance and low host specificity (Torrecillas et al., 2012). Furthermore, Rosendal et al., 2009 reported *Glomus mosseae* as commonly found in agricultural fields. Dai et al 2013 also reported the same species as common in cultivated prairie soils. This cosmopolitan species has been reported in all continents except Antarctica (Rosendal et al., 2009).

In our study we also found that, *R. verrucosa* and *Glomus ambisporum* were more abundant in natural forest soils. The findings corroborate those of Jefwa et al., 2012 who reported the similar findings on the occurrence of AM fungi across seven different land use types in Kenya. In their study, *Glomus ambisporum* was more abundant in indigenous forests than in maize and horticultural croplands. Likewise *R. verrucosa* spores were found to be more abundant in non-cropped systems with little or no antropogenic interference. Furthermore, species belonging to the family Gigasporaceae were generally more dominant in the natural forest compared to croplands. The spore density of *Gigaspora margarita* was highest in natural forest soils and interestingly *Scutellospora pellucida* a member of the same family was detected only in the natural forest sites. Our findings concur with those from studies in Venezuela and Indonesia that indicated absence of genera Gigaspora and Scutellospora upon soil disturbance imposed through agricultural practices with shifts in AM fungal communities in disturbed soils resulting in members of Glomus and Acaulospora being more dominant (Selvam and Mahadevan 2002; Cuenca et al., 1998; Boddington and Dodd, 2000).

According to Jefwa et al., 2012 land use types under cultivation was associated with lower spore abundance in Gigaspora and Scutellospora. Several other studies have reported decreases in AMF total spore abundance associated with the application of inorganic fertilizers. The findings suggest that the species in the two genera are highly sensitive to agricultural management practices.

Diversity of AMF species is measured mainly by extracting, counting and identifying field collected asexual spores, the fungal propagules that possess molecular characters to define species in this group of organisms (Leal et al., 2009; Morton et al., 1995). In our study, we used morphological characterization of field collected spores to measure the diversity of AM fungi. However, molecular techniques which have been revealed as useful tools for characterization and identification could further aid in understanding community composition and structure across different land use categories. An additional approach which uses trap cultures using bulk soils represents another strategy to yield large numbers of healthy AMF spores which can be readily identified and supplement the assessment of local species diversity across different land use types. However this methodology does not necessarily allow the identification of all species in the original bulk soil, since sporulation of the fungal community is often affected by the choice of the host plant for the trap cultures and in other cases it may promote the sporulation of cryptic AMF species that were not sporulating under field conditions (Leal et al., 2009; Sturmer, 2004). However, a panel of investigations using a combination of the above techniques may further aid our understanding of community assemblages in diverse ecosystems.

CONCLUSION

Our study revealed significant variation in AMF fungal community species composition and structure based on AMF spore characteristics under different land use regimes. Remarkably, measures of species diversity clearly distinguished land use under cultivation and non-cultivation, providing evidence that for shifts in AMF community assemblages with changes in land use practices from native forest to croplands. Contrary to ecological predictions, we found no evidence of a negative effect of crop production on the taxonomic diversity of AMF as observed in croplands in the study. However, our study showed a significant negative influence of agricultural intensification on AM fungal spore abundance and community structure. Further research should investigate whether the different AM fungal assemblages differ in their functional traits and their implications to agronomic importance.

APPENDIX

Table 3.1. Soil characteristics (0-10 cm depth) in three land use types in Machinga Forest Reserve, Machinga, Malawi, Southern and Eastern Africa

Land use type	**Soil Parameter**	**pH**	**Bulk density (Mg m $^{-3}$)**	**SOC** (g kg^{-1})
Forest reserve		6.77 (0.15)	1.22 (0.02)	11.9 (1.0)
Monoculture maize		6.64 (0.19)	1.37 (0.02)	8.3 (0.8)
Maize –Pigeon Pea Intercrop		6.78 (0.20)	1.40 (0.01)	9.2 (0.6)
ANOVA	*p value*			
Land use type (LUT)		NS	<0.0001	0.0074
Site (S)		NS	NS	NS
LUT x S		NS	NS	NS

Means with standard errors in parenthesis
NS = non-significance ($\alpha = 0.05$)

Table 3.2. Arbuscular Mycorrhizal Fungal species recovered from three land use types in Machinga soils (0-10 cm)

		Land use type		
Family	**AMF species**	**MM**	**MP**	**NF**
Glomeraceae				
	Glomus constrictum	+	+	+
	Glomus aggregatum	+	+	-
	Glomus mosseae	+	+	+
	Glomus ambisporum	+	+	+
	Glomus etunicum	-	+	-
	Funneliformis geosporum	+	+	-
	Sclerocystis rubiformis	+	+	-
	Sclerocystis clavispora	+	+	-
	Racocetra verrucosa	+	+	+
Acaulosporaceae				
	Acaulospora spinosa	+	+	+
	Acaulospora rehmii	+	+	-
	Acaulospora longula	+	+	+
	Acaulospora denticulata	+	+	+
Archeosporaceae				
	Archeospora trappei	+	+	+
Claroideoglomeraceae				
	Claroideglomus etunicum	+	+	-
Gigasporaceae				
	Gigaspora margarita	+	+	+
	Gigaspora gigantea	-	+	+
	Scutellospora cerradensis	+	+	-
	Scutellospora dipurpurascens	+	+	-
	Scutellospora pellucida	-	-	+

+ denotes presence of the species and - denotes absence of the species

Table 3.3. α -diversity of arbuscular mycorrhizal fungi (AMF) for 0-10 cm depth in October 2014 in different land use types in Machinga Forest Reserve

Land Use type	**Shannon-Weinner**	**Simpson**
Monoculture maize	2.14 ± 0.07	0.87 ± 0.01
Maize-Pigeon pea	2.31 ± 0.03	0.85 ± 0.01
Woodlands	2.00 ± 0.03	0.84 ± 0.01
ANOVA	*p value*	
Land use type (LUT)	<0.0001	0.0014
Site (S)	NS	NS
LUT x S	NS	NS

Data are means ± Standard Error (SE)
NS = non-significance (α = 0.05)

Table 3.4. Pearson-correlation matrix for edaphic variables associated with AMF spore density in three different land use types in Machinga District, Southern Malawi (0-10 cm soil depth)

	Soil pH	BD	SOC	SIL	CLY	SND	SD
Soil pH	-						
BD	**-0.02**	-					
SOC	**0.04**	**-0.29**	-				
SIL	**0.01**	**-0.70*** **	**0. 37***	-			
CLY	**0.02**	**-0.11**	**0.05**	**0.23**	-		
SND	**-0.07**	**0.32**	**-0.18**	**-0.51**	**-0.93*** **	-	
SD	**-0.11**	**-0.47****	**0.19**	**0.44****	**0.21**	**-0.30***	-

BD: Bulk density; SOC: Soil Organic Carbon; SIL: Silt; CLY: Clay; SND: Sand;
SD: Spore Density
An asterisk (*) signifies a difference at $p < 0.05$
(**) signifies a difference at $p < 0.01$
(***) signifies a difference at $p < 0.0001$

Table 3.5. Diversity measures used to describe communities of arbuscular mycorrhizal fungi (AMF)

Species richness	Measured as species density (number of species / specified area)
Shannon-Weiner index of diversity (*H´*)	$H' = - \sum P_i \ln P_i$
Evenness (*E*)	$E = H' / H_{max}$
Biovolume (Biovol)	$\text{Biovol} = 4/3\pi r^3$
Simpson's index (*D*)	$D = \sum [n_i (n_i - 1)N(N-1)]$
Modified Berger-Parker index (*d*)	$d = \text{Biovol}_{max} / \text{Biovol}_{total}$

Table 3.6. Diversity measurements of AMF communities in different land use types (0-10 cm soil depth) in Machinga, Southern Malawi

Land use type	**Description**	Richness	Evenness (*E*)	Biovolume (Biovol)	MBP (*d*)
Monoculture maize	Crop land	17	0.77 ± 0.03	0.60 ± 0.19	0.08 ± 0.03
Maize-pigeonpea intercrop	Cropland	19	0.80± 0.01	0.40 ± 0.09	0.15 ± 0.04
Natural Forest	Miombo woodlands	11	0.85± 0.01	0.67± 0.18	0.11± 0.03
ANOVA	*p value*				
Land use type (LUT)		<0.0001	0.0023	NS	NS
Site (S)		NS	NS	NS	NS
LUT x S		NS	NS	NS	NS

Data are means ± Standard Error (SE)
NS = non-significance ($\alpha = 0.05$)

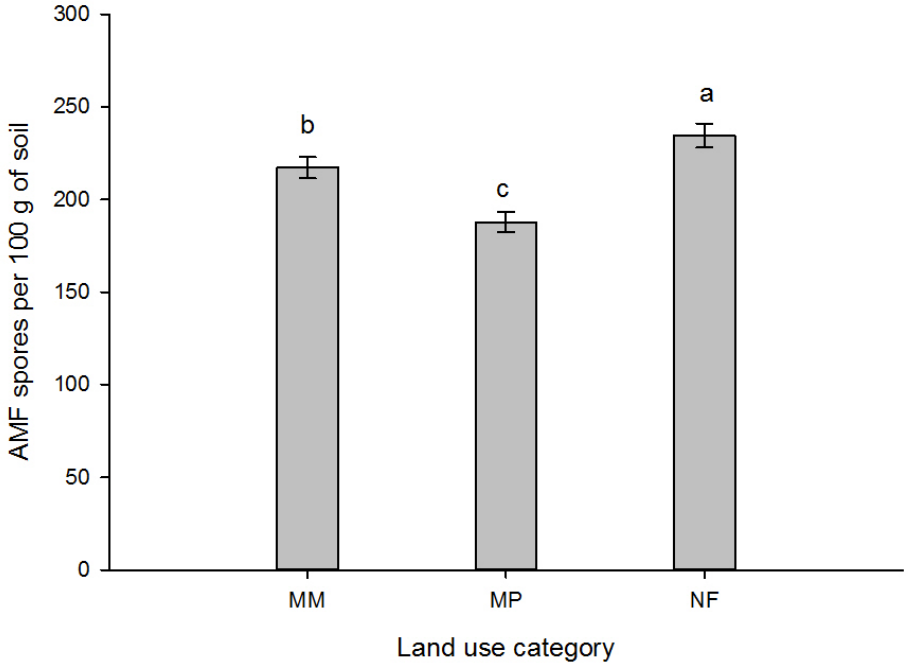

Figure 3.1. Mean AMF spore density in soils sampled from different land use categories in Machinga Forest Reserve, Southern Malawi. Error bars represent standard errors (SE). Different letters denote significant differences ($p<0.05$).

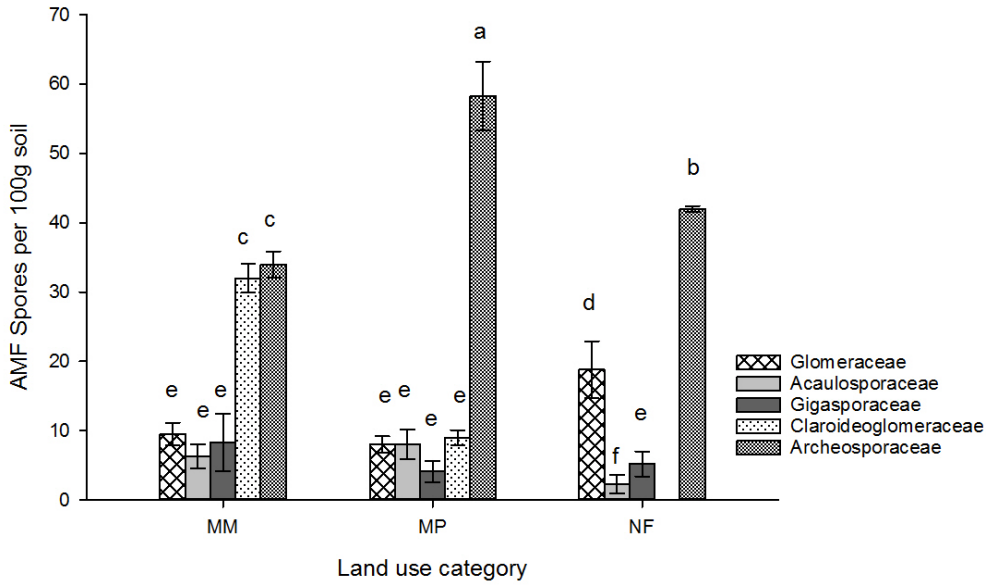

Figure 3.2. Mean AMF spore density in soils sampled from different land use categories in Machinga Forest Reserve, Southern Malawi. Error bars represent standard errors (SE). ($p<0.05$). Different letters denote significant differences ($p<0.05$).

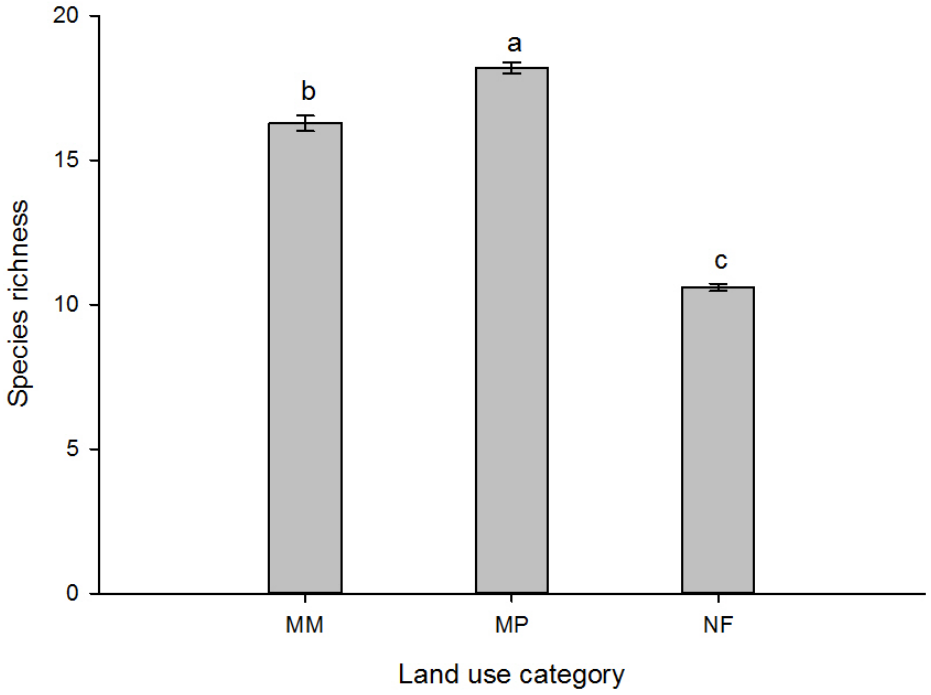

Figure 3.3. Species richness of AMF communities in different land use categories in Machinga Forest Reserve, Southern Malawi. Error bars represent standard errors (SE). ($p<0.05$). Different letters denote significant differences ($p<0.05$).

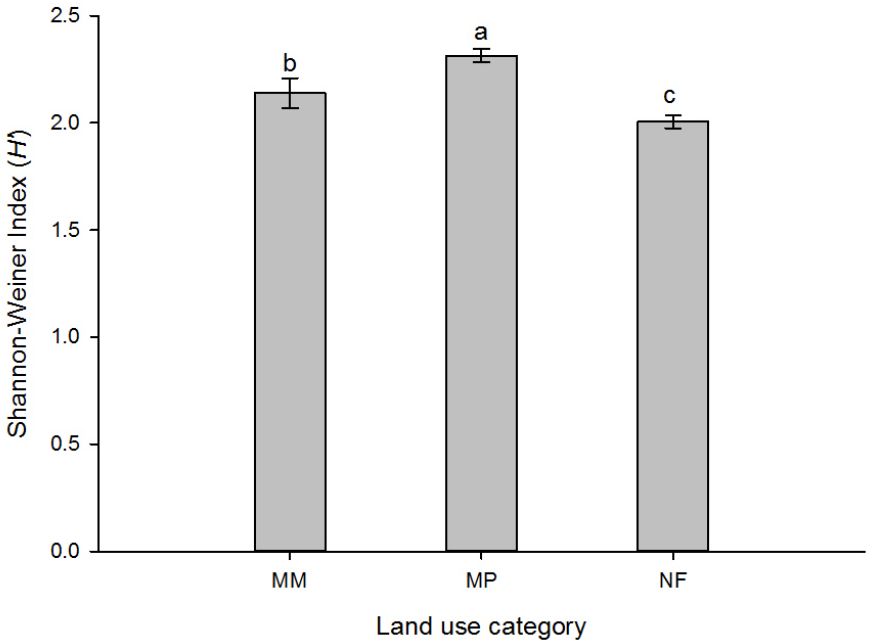

Figure 3.4. Shannon-Weiner index of AMF in three different land use categories in Machinga Forest Reserve, Southern Malawi. Error bars represent standard errors (SE). ($p<0.05$). Different letters denote significant differences ($p<0.05$).

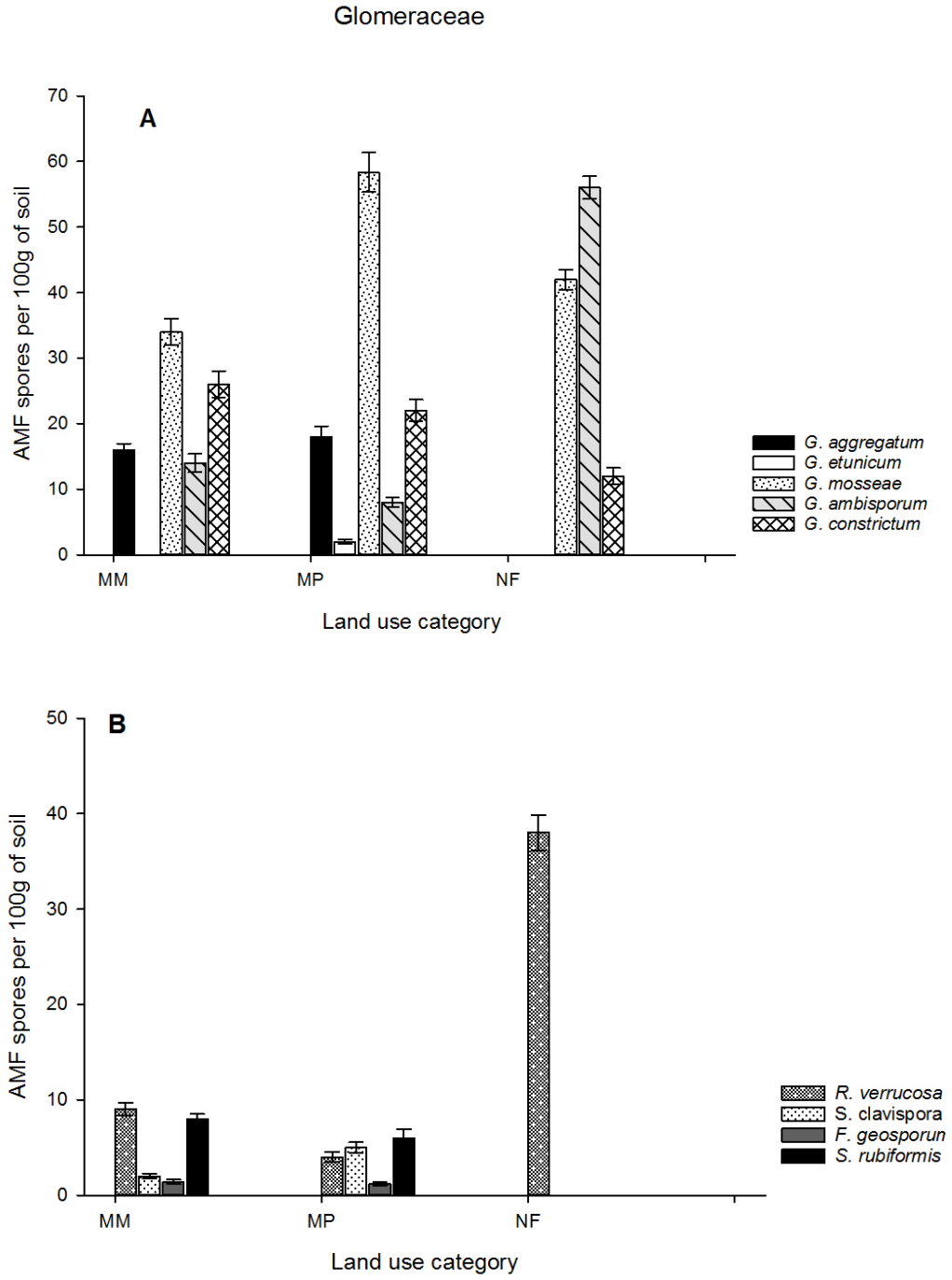

Figure 3.5. Mean spore density of species in the genus *Glomus* (A) and three other genera *Racocetra, Funneliformis* and *Sclerocystis* (B) of the family Glomeraceae recovered from three land use categories in Machinga Forest Reserve, Southern Malawi

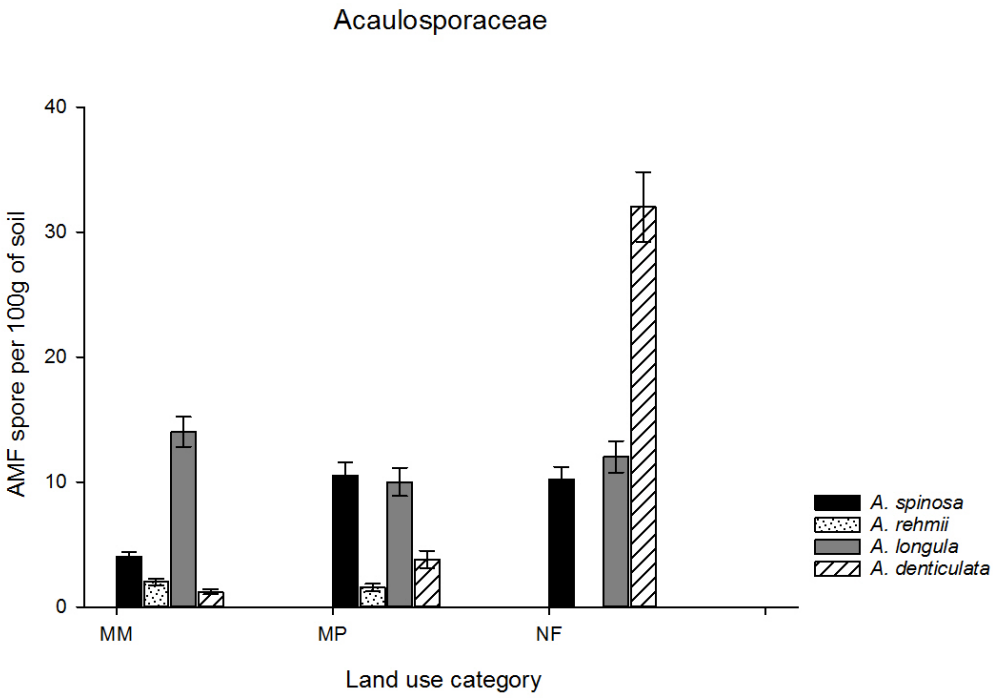

Figure 3.6. Mean spore density of species in the family Acaulosporaceae recovered from soils in three land use categories within Machinga Forest reserve, Southern Malawi.

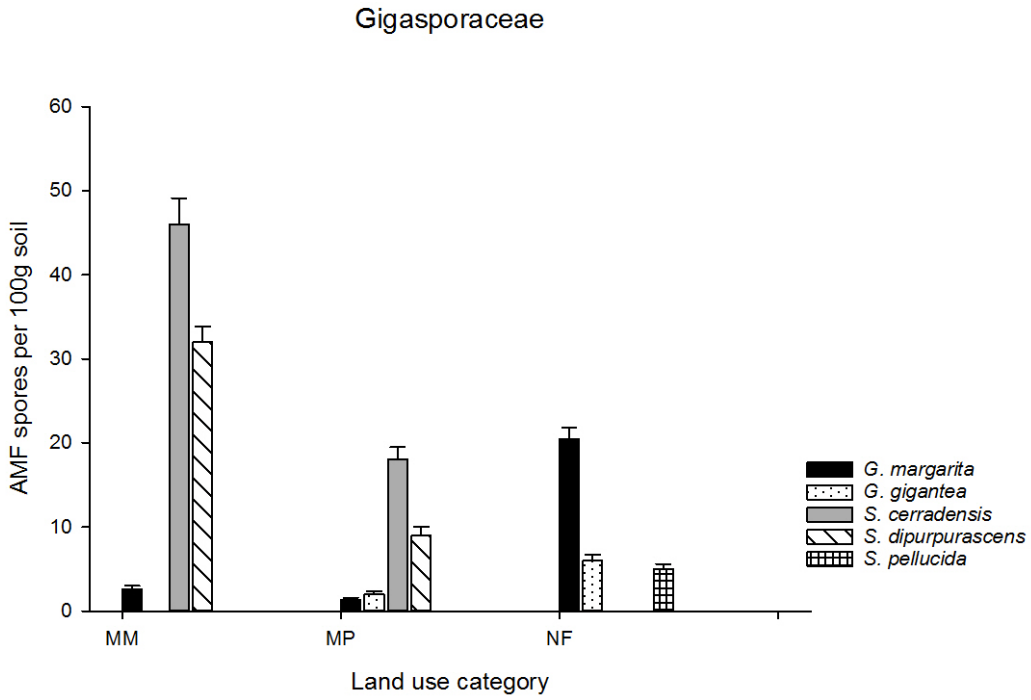

Figure 3.7. Mean spore density of species in the family Gigasporaceae recovered from soils in three land use categories within Machinga Forest reserve, Southern Malawi.

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CHAPTER 4

QUANTIFYING SPATIAL DISTRIBUTION AND VARIABILITY OF SOIL ORGANIC CARBON IN MACHINGA DISTRICT, SOUTHERN MALAWI

ABSTRACT

Detailed maps of Soil Organic Carbon (SOC) spatial distribution of are necessary to guide sustainable land use and management decisions at various scales. SOC mapping is essential in both global climate change research and food production systems at farm level, yet most studies on SOC distribution pertain to temperate climate agriculture, and the unknowns in tropical regions, particularly Sub-Saharan Africa, remain massive. In this study, we selected Machinga District in Southern Malawi, Sub-Saharan Africa to quantitatively determine SOC distribution at landscape scale and evaluate SOC prediction accuracy of a suite of various geostatistical interpolation techniques. Our study explored temporal SOC changes over a period of 2 decades, using geostatistical approaches. Mean SOC concentration on surface layer soils (0-30cm) was 8.5 g kg^{-1} soil in 2013. SOC concentration was significantly correlated with land use type, NDVI, slope and pH. SOC predictions with ordinary kriging were more accurate than those obtained using other interpolation techniques. This study provides important contributions on spatial variability of SOC in Malawi to guide land management policy, provides crucial implications of SOC management in tropical Africa to the global carbon cycle. Our findings highlight the need for rehabilitation of SOC as an integral goal of agricultural land management policy in Machinga.

INTRODUCTION

Current concerns about climate change and global warming have created an urgent need for carbon accounting at local, national, regional and global levels. Thus SOC has received increasing attention worldwide mainly because of its important role in in the global C cycle and its potential feedback on global warming (Zhang and Shao, 2014). As one of the largest and most dynamic component in the global C cycle, the SOC stock is estimated as at least twice the amount of C stored in vegetation and the atmosphere combined (IPCC,2000). Consequently, a small loss in of the SOC pool due to changes in land use, cropping systems, farming practices and soil erosion can have far reaching impacts in increasing the atmospheric CO_2. On the other hand, soils can also act as carbon sinks and increase the existing SOC pool by sequestration of atmospheric C, the processes of which are an active area of study. Reliable assessment of the spatial patterns and SOC spatial distribution as baselines are essential for understanding factors that control SOC spatial patterns and changes in global climate change research.

The potential of soils to sequester C presents an opportunity for climate change mitigation, underscoring the urgent need for investigating drivers that control SOC sequestration and maintenance at regional and local scales. In addition, such studies provide the means for identifying SOC sink and source capacities in changing the environments which is useful in informing policy direction and the development of effective climate change and global warming mitigation strategies. Numerous studies have been conducted on SOC spatial distribution but they largely pertain to temperate climate agriculture, whereas the unknowns in tropical regions, particularly Sub-Saharan Africa, remain massive (Diels et al, 2004).

Farming systems in sub-Saharan Africa are mostly smallholder systems with very little or no use of inputs. Because of the differences in soil mineralogy, climate, types of organic matter inputs and other factors between temperate and tropical systems, controls of SOC stability and consequently its spatial distribution are likely slightly or drastically different, precluding a straightforward transfer of concepts generated from research on temperate systems (Torn et al., 1997).

There are additional reasons why quantifying SOC content is particularly important in sub-Saharan Africa. The region is characterized by ever-increasing human demands on soil derived ecosystem services. Soil organic carbon is an important determinant of physical, chemical and biological properties of soil and is critical for improving soil fertility and quality, increasing the water holding capacity of soil, reducing soil erosion, improving soil structure and enhancing crop productivity (Zhang and Shao, 2014). It is particularly important as the single largest source of nutrients in smallholder farming systems of Southern Africa (Beedy, 2009).

How to build up and maintain soil fertility is a crucial issue in increasing agricultural productivity and achieving food security in Africa (Beedy, 2009). Detailed soil organic carbon maps showing spatial SOC distribution are necessary to guide sustainable land use and management decisions from farm to catchment, national and regional scales. However, information on SOC pools and SOC distribution both spatially and temporally, is in many cases not available.

This is particularly true in sub-Saharan Africa constrained by many developmental challenges, limiting the capacity of evaluating SOC stocks and SOC distribution and as well as the prediction of ecosystem response to environmentally and anthropogenically induced changes.

Two major analytical SOC determination approaches exist, namely wet chemical oxidation method and the dry combustion with automated elemental analyzers (Chen et al., 2015). The choice of an approach for SOC determination is determined by a number of factors including the reliability, reproducibility, time-efficiency, cost of equipment or chemicals and the possible environmental risk (Lettens et al., 2007). Both approaches have been widely used to measure SOC content over the past 60 years. The Walkley-Black method is a wet chemical oxidation method that is rapid and requires less equipment compared to other wet or dry combustion methods (Nelson et al., 1982). The Walkley Black method has been the most widely reported procedure for the past several decades. Nevertheless, this approach may lead to widely variable recovery of SOC and brings the risk of using the hazardous chromium-containing dichromate (Chen et al., 2015). In contrast, the dry combustion method using an automated combustion analyzer has been increasingly used in many parts of the world owing to its simplicity and accuracy and environmentally friendly nature, despite the higher expense of the analyzer and consumables. Indeed, it has been proposed that automated dry combustion is the only reliable, comprehensive method to determine soil C concentration with the added benefit of simultaneous measurement of Nitrogen and Sulfur (Chatterjee et al., 2009; Chen 2015). The Walkely Black method is therefore being progressively replaced by more accurate dry combustion analyses in many countries.

When evaluating the change of SOC stocks over time where absolute SOC assessments are required, an important issue arises as to how to correctly interpret historic soil analytical results, which most often were obtained by methods based on Walkley Black method measurements. One approach to overcome this challenge is to apply a correction factor to generate comparable results. Numerous studies comparing results obtained by the two methods have been conducted in many parts of the world. However, deduced conclusions about the correction factors have not been decisive (Gelman et al., 2011).

This study focused on Machinga district which provided opportunities to evaluate SOC stocks in a landscape noted for its variable geographic nature and distinct land use types. The objectives of this study were: (1) to quantify SOC content across land use types in Machinga disticrict; (2) to assess changes in SOC content attributable to land use changes over a period of two decades; (3) to determine the optimal interpolation method, that is suited to the hilly–gully and wetland terrain as exemplified by Machinga in sub-Saharan Africa.

MATERIALS AND METHODS

Study area

The study area is located in the Southern region of Malawi, a country in sub-Saharan Africa. Malawi lies within the tropics between latitudes 9° S and 18° S and longitudes 32° E and 36° E. The country is divided into 28 districts within three administrative regions namely northern, central and southern region. Machinga district lies in the southern region at 14 ° 58' 00'', 35° 31' 00" E and covers an area of 3,771 km^2. The area ranges in elevation from 800 m in the Lower Shire valley to 1300 m in the upper Shire valley. Temperatures averages 31.6° C in November and 17.0° C in June. The climate is semi-arid, with an average annual precipitation of 800 to 1300 mm (Quinion, 2008). There is one primary rainy season between November and April, but intermittently there may be a period of light rains called 'chiperonis' during May, June and July (Msuku et al., 2005).

Vegetation is characterized by lakeshore savanna grassland and thickets in the Upper Shire Valley, Semi-arid savanna grassland and thickets further south, Miombo woodlands and semi-evergreen forests in Malosa, Chikala, Chinduzi and Liwonde forest reserves. The dominant tree species in the forest reserves are *Branchystegia boehmii, Burkea Africana, Bridelia micrantha, Pericopsis angolesis* and *Pterocarpus angolesis.*

Perennially wet grasslands are located to the east bordering Lake Chilwa and Lake Chiuta (Figure 1) while open canopy woodlands and shrubs are mostly located in upland areas and in the Kawinga plains.

Soils are predominantly ferrallitic (ferrasols) (Lowole, 1993). The ferrallitic soils have a sandy loam top soil and low inherent fertility, and are generally classed as Alfisols and Ultisols in US *soil taxonomy* (Soil Survey Staff, 1975), with some presence of Vertisols in low lying drainage areas locally termed 'Dambos'. The low-fertility, sandy and coarse textured soils make households in Machinga especially vulnerable due to inherent poor crop production potential and nutrient depleted status (MVAC, 2005).

Land Use Types

Forest areas

Machinga District has a forest coverage estimated at 92,265 ha which represents 16% of the total land area. Forests in the district exist in several categories ranging from gazetted forest reserves, wildlife reserves, government plantations, privately owned plantations, individual woodlots, communal forests and village forests (Machinga SEP, 2012). The most common vegetation in the study area which covers the gazetted forest reserve of Machinga are the *Brachystegia* woodlands, and *Eucalyptus*. Comparatively the *Brachystegia species* occupies the highest area of forest cover in the district (Machinga SEP, 2012).

Agricultural land

Agricultural activities take place on 217,322 hectares, which comprises 57 % of the land mass. Machinga district has high, medium and low agricultural potential areas. Nearly 80% of the district's total landmass is classified as arable land but only 44% of the arable land is of high agricultural potential , 46% is considered marginal and the rest is arable land of low agricultural potential (Machinga SEP, 2012).

Lakes

Of the total land area in the district, 0.3% is occupied by Lakes Chiuta, Chilwa and Malombe (Fig. 1). The remainder of the land is wetlands and human settlement, forest reserves and agricultural fields.

Sampling and measurement methods

A set of 250 soil samples were collected from Machinga District from a total of 84 locations following baseline soil sampling sites from a country wide survey conducted from 1987-1990 in Malawi (Land Husbandry Branch, Ministry of Agriculture, Government of Malawi, 1991; Venema, 1990). The baseline involved georeferenced site evaluations, including excavating soil pits and comprehensive observations on topography, land use, and soil pedagogy. Soil chemical analyses were also conducted on soil profile layers which varied from 2 to 4 layers, depending on profile characterization of the soil pits. The data collected included: soil parent material, physiographic unit, altitude, macro and micro topography, slope description (gradient, length and slope position), erosion present, permeability and drainage class, vegetation, land use and FAO soil classification. Land use descriptions with information on the plant species present (including crops for agriculturally-used land, and dominant plant species for natural areas), were also recorded. The vegetation and land use observations that were made at the sites were linked to land cover photo interpretation to create a 1:250,000 scale land use and land cover map output.

In this study, a Garmin Etrex 12 Channel – Global Positioning System (GPS) receiver was used, each site located and data on elevation recorded. The terrain information, land use and additional information was also recorded. Before collection, the surface litter was removed at the sampling spot and then a soil pit was dug using a hand hoe to a depth of 30 cm.

The samples thus collected were thoroughly mixed and checked for foreign materials. A desired quantity of the composite was obtained by taking one fifth of the total composite sample and the sample so collected put into a clean labeled polythene bag. The samples collected were air dried, sieved with a 2 mm sieve after 7 days of air drying and powdered with mortar and pestle. Soil material obtained was analyzed for important physical and chemical properties by following the standard procedures. All samples for this study were collected from September to October, 2012. Soil chemistry analyses were conducted at Chancellor College, Department of Biological Sciences Soil Fertility Research Laboratory in Zomba and The W.K Kellogg Biological Station, Long Term Ecological Research Station (KBS LTER) facilities in Michigan, U.S.A. Measurements conducted at Chancellor College included include soil texture, pH (in water, 2:1 ratio), organic C, cations (K, Ca and Na). Soil texture determination was by the hydrometer method where the settling rates of primary particles are based on the principle of sedimentation as described by Stokes'Law (ASTM 152H-Type hydrometer) (Gee and Bauder, 1986).. Combustion method was used for C and N determination, after grinding a subsample to pass a 1-mm screen, using a Costech Elemental Combustion Analyzer (ECS4010, Valencia, CA).

Soil Organic Carbon (SOC) and Soil Nitrogen measurements

Soil organic carbon analyses for the baseline samples were conducted at the Soil Commodity Team Laboratory, Chitedze Research Station, Ministry of Agriculture, Government of Malawi, using the internationally recognized Walkley Black method (wet combustion of the organic matter with a potassium dichromate/sulphuric acid mixture and titration of the residual dichromate with ferrous sulphate. Calorimetric quantification of the organic carbon present was then performed.

Organic matter content was then derived from the total organic carbon by the following formula. On the other hand, total nitrogen for the baseline samples was quantified using Kjeldal method whereby a 40-mesh sieved soil sample was digested with concentrated sulphuric acid. The digest was then distilled and the distillate, containing ammonium nitrogen, was titrated against a weak hydrochloric acid solution (HCl). The amount of HCl used in the titration was then used to calculate the amount of nitrogen in the sample using correlation (Eschweiler et al., 1991). Owing to the reliability and environmentally friendly nature of the automated dry combustion method, the current study used this approach. Soil organic carbon and total nitrogen content were determined using a Combustion analyzer at KBS LTER. To compare the historic SOC status with the present data, a correction factor was used to convert the historic SOC values using published Walkley Black correction for tropical soils (Dieckow et al, 2007)

$C = 1.05_{WBlack} + 0.47$ **(1)**

where;

C = Corrected SOC value derived from baseline dataset

WBlack = baseline SOC value obtained by Walkely Black method

Furthermore, SOM content was evaluated from the organic carbon data from both the baseline and the current study using the following formula:

SOM = OC × 1.724 (Eschweiler et al., 1991; Venema, 1990) **(2)**

where

SOM = Soil Organic Matter

OC = Organic carbon

SOM status of the sampled area was classified into four different levels (*i.e.*, very low, low, medium and high) based on the USDA textural classes. Topographic factors were computed using a spatial analysis model and digital topography analysis, and included elevation (H), slope (b), sinα and cosα of aspect, compound topographic index (CTI) and stream power index (SPI).

Data mapping optimization and validation using geostatistical approaches

A descriptive statistical analysis was used as the initial step to explore the central trend and the overall variation of different variables under investigation. The analysis included description of the minimum, maximum, mean median, skewness, Kurtosis, Standard deviation and the coefficients of variation (CVs). A one sample Kolmorogorov –Sirminov (K-S) test was used to examine the normality of the data and natural logarithmic transformations were performed where necessary to meet the normality requirement of geostatistical analysis (Zhang and Shao, 2014).

Modelling of spatial variability and the estimation at unsampled locations were performed using geostatistical approach. Using two-dimensional space, the spatial structure of a variable can be visualized by maps. However, in order to produce spatial representation of dataset, interpolation of the values at unsampled locations is necessary. Various interpolation techniques exist (*e.g.*, inverse distance weighting, IDW, also termed inverse distance to a power, triangulation with linear interpolation) which are exact interpolators, however, such methods do not take into consideration the spatial autocorrelation of data and thus oversimplify the reality (Isaaks and Srivastava, 1989; Robinson and Metternicht, 2006; Mabit *et al*., 2008, Marchetti et al., 2012).

Consequently, Ordinary Kriging (OK), known as the best linear unbiased estimator (BLUE), was chosen as interpolation method for each soil parameter to minimize the prediction error variance. OK is by far the most common type of kriging, consisting in a form of weighted averaging, and is based on the concept of a variable *Z(x)* that is both random and spatially autocorrelated (Heuvelink and Webster, 2001). The predictions are based on the following model:

$$\mathrm{Z}(x) = \mu + \varepsilon(x) \quad \textbf{(3)}$$

where μ is the constant stationary function (global mean)

$\varepsilon(x)$ is the spatially correlated stochastic part of variation.

Estimation of *Z* at an unsampled point x_0, $\hat{Z}(x_0)$, is made by a weighted average of the data

$$\hat{\mathrm{Z}}(x_0) = \sum_{i=1}^{n} \lambda_i \mathrm{Z}(x_1) \quad \textbf{(4)}$$

where λ_i is the kriging weight assigned to sampling site $Z(x_i)$. The weights are allocated to the sample data within the neighborhood of the point to be estimated in such a way to minimize the estimation variance. To ensure that the estimate is unbiased, weights are made to sum up to 1.

$$\sum_{i=1}^{n} \lambda_i = 1 \quad \textbf{(5)}$$

and the expected prediction error computed as;

$$E\left[\hat{\mathrm{Z}}(x_0) - \mathrm{Z}(x_0)\right] = 0 \quad \textbf{(6)}$$

Semivariances are then used to estimate weights objectively, so that they reflect the true spatial autocorrelation structure using the formula below:

$$\gamma(h) = \frac{1}{2} E\,[\mathrm{Z}\,(x_1) - \mathrm{Z}\,(x_1 + h)]^2 \qquad \textbf{(7)}$$

where;

Z (x_i) is the value of the target variable at sampled location i and Z (x_i +h) is the value of the neighbor at distance h. A set of n point observations yields n (n − 1)/2 pairs for which a semivariance is calculated.

A semivariogram model contains three crucial parameters which interpret the spatial structure of soil properties: nugget (C_0), sill ($C+C_0$), and range (A). Nugget represents the undetectable measurement error, inherent variability or the variation within the minimum sampling distance. Sill is the upper limit of the semivariogram model, representing the total variation. The separation distance at which the sill is reached is the range of spatial dependence. Samples separated by distances smaller than the range are spatially related, whereas samples separated by larger distances are not spatially related. The nugget ratio (C_0/C_0+C) can be regarded as a criterion for classifying the spatial dependence of soil properties.

A variable is considered to have strong, moderate, or weak spatial dependence if the ratio is less than 0.25; between 0.25 and 0.75; and over 0.75, respectively (Zhang and Shao, 2014). After selecting the best-fit semivariogram models, ordinary kriging was used as an interpolation method to predict values for SOC content.

Cross validation procedure was conducted to evaluate the accuracy of the model using three statistical measurements of prediction error: mean error (ME), root mean square error (RMSE), and root mean square standardized error RMSSE (Marchetti et al., 2012, Zhang and Shao, 2014) as indicated below:

Mean error:

$$\text{ME} = \frac{1}{N}\sum_{i=1}^{N} [\hat{\text{Z}}(x_i) - \text{Z}(x_i)] \quad \textbf{(8)}$$

Root mean square error:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} [\hat{\text{Z}}(x_i) - \text{Z}(x_i)]^2}{N}} \quad \textbf{(9)}$$

and root mean square standardized error:

$$\text{RMSSE} = \sqrt{\frac{\sum_{i=1}^{N} \{[\hat{\text{Z}}(x_i) - \text{Z}(x_i)]/\sigma(x_i)\}^2}{N}} \quad \textbf{(10)}$$

where $\sigma(x_i)$ is the prediction standard error in location x_i.

R_I values were used to evaluate improvements in the prediction accuracy by comparing the various interpolation techniques to ordinary kriging (Batjes and Sambroek, 1997; Guo and Gilford, 2002).

$R_I = \frac{RMSEok - RMSE}{RMSEok}$ x 100%

where;

R_I is the improvement in prediction accuracy

RMSEok is the root mean square prediction error of ordinary kriging

RMSE is the root mean square prediction error of the interpolation technique being compared.

Statistical Analysis

Descriptive statistical analysis was used to explore the central trends and overall variation of variables of interest. The analysis included descriptions of the minimum, maximum, mean, median range, standard deviation and coefficients of variation (CVs). The normality of the data was examined using a one sample Kolmogorov-Smirnov (K-S) test and where necessary natural logarithmic transformations were performed to meet the normality requirements of geostatistical analysis (Zhang and Shao, 2014). SAS 9.4 (2002-2012) by SAS Institute Inc., Cary, NC, USA was used for statistical analysis of the soil characteristics including pH, SOC, sand, silt and clay content in Machinga District, Southern Malawi. Geostatistical analyses and GIS mapping were conducted with ArcGIS 10.2 with the Geostatistical Analyst extension by ESRI, 380 New York Street, Redlands, CA 92373-8100.

Topographic factors were extracted from the Digital elevation model (DEM) of Machinga district set in ArcGIS 10.2. For a given variable all kriged maps were kept on the same scale in order to allow easier comparisons. Means across different land use categories were compared using one way analysis of variance (ANOVA). Stepwise linear regression was carried out in SAS 9.4 using PROC REG procedure to explore relationships between SOC and other soil characteristics as well as terrain attributes.

RESULTS

Descriptive statistics of SOC and other variables in Machinga District, Southern Malawi

Mean SOC content in Machinga in 2013 was 8.5 g kg^{-1} with a coefficient of variation 59.1% (Table 4.1), reflective of the complex topographical variability and the variable nature of Machinga district (Figure 4.1). Soil pH ranged from 4.9 to 8.3 with a coefficient of variation of 12.5% and a mean of 6.5 (Table 4.1) for the same year. Baseline data from the same sites in 1990 indicates that SOC values averaged at 8.9 g kg^{-1} with a coefficient of variation of 55.5%. The pH in 1990, ranged from 4.9 to 7.7 with a coefficient of variation of 11.6 % and a mean of 6.2 (Table 4.1). Our findings indicate that overall, SOC values have decreased by 4.7 % over the two decade period in the district. The results showed that silt and clay content were generally more variable compared to sand throughout the study area (Table 4.2). The CVs of sand, silt and clay were 23.7%, 70.0% and 83.6 % respectively. Sand content ranged between 5.3 – 93% with a mean sand content of 73.6%, silt content ranged between 1.0 – 19.0 % with a mean value of 7.3% and clay content ranged between 3.0 – 87.0 % with a mean value of 19.1% (Table 4.2).

Vertical distribution of SOC in Machinga District

The distribution of SOC was significantly influenced by depth ($p<0.0001$). Overall vertical distribution of SOC in Machinga district generally varied with mean SOC concentrations of 19.2 g kg^{-1} , 11.9 g kg^{-1}, 8.6 g kg^{-1}, 7.8 g kg^{-1} for the four descending depths profiles (0-5 cm, 5-10 cm, 10-15 cm and 15 -20 cm respectively) (Figure 4.2). The results indicated that the SOC content was progressively decreasing with soil depth and that the magnitude of the differences were more pronounced particularly with the four upper depth profiles (Figure 4.2). Our results also indicated that the vertical distribution of SOC was also influenced by land use type (Figure 4.3 A-E).

Taking depth and land use types as major determinants of vertical distribution of SOC, results indicated that SOC content was highest in the upper depth (0-5 cm) across all land use types (Figure 4.3A-E) and generally progressively decreased along the depth profiles for all land use types. In the uppermost depth profile (0-5 cm), SOC content varied in the order wetlands > rangelands > forest for unmanaged ecosystems (Figures 3 C-E). On the other hand, SOC content in the managed ecosystems followed the order conversions > croplands (Figure 4.3 A-B). By contrast, managed ecosystems registered comparatively lower SOC levels for the uppermost depth profile.

Correlations of SOC with environmental variables

Over the two decades, gains in SOC content in the topsoil were attained in unmanaged ecosystems (forest, wetlands and rangelands) compared to cultivated areas or managed ecosystems (croplands and conversions) (Figure 4.5). Results of stepwise regression of different physico-chemical factors as well as terrain attributes indicated that SOC was significantly correlated with Normalized Difference Vegetation Index (NDVI) ($r=0.430$, $p<0.01$, Table 4.3). Soil organic C was found to be positively correlated with above ground productivity as indicated by the positive relationship. NDVI measures the vegetation cover and our findings indicate that increases in vegetation cover are associated with increases in mean SOC concentration in Machinga district. Slope and soil pH also influenced SOC content in Machinga district ($p<0.05$, correlation coefficients of 0.287 and 0.252 respectively) but to a lesser extent. The association indicated that increase in pH was associated with increases in SOC content. Likewise increases in slope were also associated with increases in SOC content.

The hilly and high slope areas are mostly covered by natural forests in Machinga. There was very little positive correlation between SOC and Stream power Index (SPI) and a weak negative correlation with Compound Topographic Index (CTI) among several terrain attributes that were investigated (Table 4.3).

SOC Changes across land use types in Machinga District

Since land use type was found to be a major driver of the spatial distribution of SOC in Machinga, we were interested to further explore if there were any temporal changes in SOC content to the depth of 0-30cm, across land use types in Machinga district over a period spanning over two decades. Our results indicate that changes in SOC content were not the same across land use type. Changes in SOC content were significantly different in lands under conversion and wetlands land use categories two decades after the baseline ($p<0.0001$, Table 4.5). Lands that were originally under fallow and had been converted to croplands (conversions), demonstrated largest SOC losses (Figure 4.5). Fallow sites had a mean SOC value of 8.1 g kg^{-1} in 1990 and after conversion the same sites registered lower mean SOC value of 5.1 g kg^{-1} in the subsequent survey. On the other hand, SOC accrual over the period spanning 2 decades was statistically significant in lands under wetland land use category. In 1990, wetlands registered a mean SOC value of 9.3 g kg^{-1} . Two decades later, significant SOC accrual occurred in wetlands to a mean value of 10.7 g kg^{-1} ($p <0.0001$, Table 5). Results indicated that SOC changes in croplands, forests and rangelands were however not significant different over the 2 decades (Table 4.5). In 1990, crop lands registered mean SOC value of 10.3 g kg^{-1} with a wide range of 3.3 – 19.0 g kg^{-1}.

On the other hand two decades later the same land use category registered a mean SOC value of 9.5 g kg^{-1} with a range of 3.2 - 15.6 g kg^{-1}. Taken together, increases in SOC content were associated with unmanaged ecosystems namely; forests, wetlands and rangelands and decreases in SOC were associated with managed ecosystems (croplands and conversions) (Figure 4.5). As indicated, the magnitude of SOC accrual was highly variable across land use types based on the base line and subsequent survey data spanning a period of two decades. Mean SOC levels across forest sites increased from 7.5 g kg^{-1} to 10.0 g kg^{-1} after twenty years (difference of + 2.5 g kg^{-1}, Figure 4.5). SOC surface maps for 2013 and 1990 revealed the changes in SOC over the period spanning over two decades (Figure 4.4A-C).

Spatial distribution of soil carbon at landscape scale: Comparison of kriging models and their prediction accuracy

Thirty five randomly selected samples were used to conduct inverse distance weighting interpolation, spline kriging, universal kriging and ordinary kriging interpolation. From the prediction error maps and distribution maps various interpolation techniques, we evaluated the prediction accuracy of each interpolation technique (Table 4.4). Our results indicated that ordinary kriging with spherical semivariogram model (OKS) was better than the rest of the techniques employed. The mean prediction error (MPE) for OKS was 0.0424 and root mean square prediction error (RMSE) was 0.2059 (Table 4.4). The ordinary kriging predictions were much more detailed concerning the partly variation and topographical relationships and much more close to the observed spatial distribution of SOC. Predicted values obtained by ordinary kriging methods were thus more accurate using than those obtained by other interpolation techniques in the study.

The improvements of prediction accuracy obtained using ordinary kriging with spherical semivariogram model are summarized in Table 4.4. Our findings indicated that the method improved prediction accuracy over inverse distance weighting, spline interpolation with using regular and tension method, universal kriging with linear semivariogram model and universal kriging with quadratic semivariogram model by 44.32%, 87.83 %, 62.50% and 35.96 % respectively (Table 4.4).

DISCUSSION

Spatial distribution of SOC and its variability in Machinga district

Controls of spatial distribution of SOC vary with scale and previous research has shown that climate and soil texture are important drivers at global scale (Jobbagy and Jackson, 2000). The importance of the same drivers has also been shown to vary when associations are examined by soil depth (Jobbagy and Jackson, 2000). Other soil related factors and plant functional types become more important in determining the spatial distribution of SOC with soil depth as an important factor. In our examination of physico-chemical characteristics and terrain attributes of the highly variable landscape of Machinga, land use type was found to be the dominant driver of SOC distribution. Similar findings were found by Peng et al., 2013 who showed that at watershed level, geostatistical characteristics of SOC concentration were closely related to land use and spatial topographic structuring. Our findings further demonstrated that both horizontal and vertical distributions of SOC were linked to land use type in Machinga district. Unmanaged ecosystems (forests, wetlands and rangelands) had higher SOC content in the upper soil layer. The observation may be attributed to several factors which include the following; 1) unmanaged ecosystems would generally fix plentiful SOC in the upper layer due to falling litter from different types of plants growing in these land use types, 2) The absence of anthropogenic disturbance such as tillage allows in unmanaged systems results in higher SOC accumulation in the upper layer of these ecosystems. On the other hand, managed ecosystems were observed to have lower SOC content probably due to SOC loss with soil disturbance. Numerous studies report about tillage and land use conversion from natural forest as associated with heavy SOC losses (Vesterdal and Leifeld, 2010; Grandy and Robertson, 2007).

According to Marchetti et al., 2012, tillage mixes upper SOC stocks with lower soil profiles rendering lower SOC stocks in the upper layers of agricultural soils in this case. Consequently, conventional tillage practices combined with non-conservative agronomic practices such as monoculture cropping causes SOC dilution in the arable soil layer due to mixing with underlying soil horizons that are poor in SOC.

Interpolation techniques for SOC spatial distribution best fitting the highly variable nature of Machinga District

A total of ten different interpolation approaches spanning across 4 main interpolation techniques were deployed using the SOC datasets to generate the average SOC surface over Machinga district. Interpolation results were compared on the basis of cross validated RMSE. As shown in Table 4.4, RMSE for different interpolation techniques are in the order OK < UK < IDW < S. Since the minimum RMSE was obtained by OK, the technique is thus an optimal method for interpolation of SOC content at unsampled locations in the district. Overall, the interpolation performance of geostatistical methods (OK and UK) was better than interpolation performance of deterministic methods (IDW and S). Our findings concur with those by previous researchers who found geostatistical methods to be better methods on estimating spatial distribution of other environmental phenomena such as precipitation in Ontario, Canada (Wang et al., 2014).

SOC Spatial distribution across space and time in Machinga district

The average SOC surfaces for Machinga created by the optimal interpolation method were used to analyze changes in SOC content over a period of two decades. The mean SOC content decreased from 8.9 g/kg in 1990 to 8.5 g/kg in 2013. Results from SOC change surface map indicated that areas to the north east and south east of Machinga registered losses in SOC.

The findings corroborate with the land use map of Machinga which shows that the trend of SOC concentration decrease followed mostly areas under arable cropping spanning from north east to south east of the district (Machinga SEP,2012). The SOC change surface map also shows that significant gains were registered after two decades from the baseline in the region southwest of Machinga. The region is mostly covered by miombo forest located in hilly areas of Machinga, and in particular, Malosa forest reserve. SOC content was observed to have increased over this region (Figure 4.4). The observation could be attributed to the fact that forest lands fix plentiful SOC because of flourishing plant roots and thicker forest litter layer (Peng et al., 2013) and that forests in hilly areas are often not preferred for conversion to agriculture unlike forest in flat areas. The Malosa forest reserve area is in contrast with the Miombo woodlands that span the western region of Machinga district where appreciable decreases in SOC content were registered over the two decades. The flat areas of this region have largely been encroached and land converted to agriculture due to their suitability for cultivation as well as population pressure.

CONCLUSION

Our study has provided for the first time spatial estimates of SOC content at landscape scale in Machinga district, southern Malawi. This is a novel exploration of fine resolution dynamics of soil C in topographically complex smallholder farming, mixed use lands. The study demonstrated spatio-temporal changes n SOC distribution and provides knowledge on the current state of SOC distribution in Machinga district which could be the basis for planning to prevent or limit, negative effects on soil properties from anthropogenic land management. Of the several interpolation techniques used in the study, geostatistical methods and in particular ordinary kriging methods improved SOC prediction accuracy, demonstrating that the approach is suitable for studying SOC spatial distribution in areas of complex topography. A novel finding of this study was that continuous cropping as well as land conversion was associated with declines in SOC. Indeed, land use type was a major determinant of topsoil SOC, and SOC change over time, but correlations were also found with NDVI, pH and slope. SOC distribution at landscape scale in Machinga district is sensitive to anthropogenic land management practices more than the other measures in the study. Practices such as conversion of fallow areas to agriculture and continuous cropping of arable lands require urgent attention if loss of SOC is to be addressed at landscape scale in mixed use lands of sub-Saharan Africa.

APPENDIX

Table 4.1. Summary results of mean concentration of Soil Organic Carbon (SOC) and pH in Machinga District

Variable	Year	Mean	Median	Min	Max	Range	Std. Dev	CV (%)
SOC ($g\ kg^{1}$)	**2013**	8.5	8.8	0.7	22.4	21.7	5.0	59.1
	1990	8.9	8.5	1.8	24.9	23.1	4.9	55.5
pH	**2013**	6.5	6.4	4.9	8.3	3.5	0.8	12.5
	1990	6.2	6.1	4.9	7.7	2.8	0.7	11.6

SOC = Soil Organic Carbon
pH = Soil pH

Table 4.2. Summary statistics of sand, silt and clay content in soils sampled in Machinga District in 2013

Variable	Mean	Median	Min	Max	Range	Std. Dev	CV (%)
Sand	73.6	78.0	5.3	93.0	87.7	23.7	23.7
Silt	7.3	6.0	1.0	19.0	18.0	4.7	70.0
Clay	19.1	16.0	3.0	87.0	84.0	15.9	83.6

Table 4.3. Pearson-correlation matrix for soil and environmental variables SOC in Machinga

	SOC	NDVI	pH	SLP	CLY	ELEV	SPI
SOC	-						
NDVI	**0.430****	-					
pH	**0.252***	**0.207**	-				
SLP	**0.287 ***	**0.250***	**-0.030**	-			
CLY	**0.030**	**-0.053**	**-0.227**	**-0.009**	-		
ELEV	**0.228**	**0.108**	**-0.080**	**0.449*****	**-0.274 ***	-	
SPI	**0.200**	**0.071**	**0.194**	**-0.023**	**-0.531 *****	**0.540*****	-

SOC: Soil Organic Carbon; LUT: Land use type; NDVI: Normalized Difference Vegetation Index; pH: Soil pH; SLP: Slope; CLY: Clay; Elev: Elevation;
SPI: Stream Power Index
An asterisk (*) signifies a difference at $p < 0.05$
(**) signifies a difference at $p < 0.01$
(***) signifies a difference at $p < 0.0001$

Table 4.4. Comparison of prediction accuracy between various interpolation techniques in Machinga District

Interpolation technique	Model	MPE	RMSE	R_I (%)
Inverse Distance Weighting	none	0.1368	0.3698	44.32
Spline	Regular	2.8631	1.6921	87.83
Spline	Tension	0.3016	0.5491	62.50
Universal kriging	Quadratic	0.1034	0.3215	35.96
Ordinary kriging	Gaussian	0.0434	0.2084	1.20
†Ordinary kriging	Spherical	0.0424	0.2059	0.00
Ordinary kriging	Exponential	0.0462	0.2149	4.19
Ordinary kriging	Circular	0.0426	0.2065	0.29
Ordinary kriging	Linear	0.0425	0.2062	0.15

MPE: Mean Prediction Error; RMSE: Root Mean Square Prediction Error; R_I: Improvement of Prediction Accuracy.
† Ordinary kriging with spherical model was used for comparison of prediction accuracy with other interpolation approaches.

Table 4.5. Summary results of mean concentration of SOC across land use types in Machinga District in 1990 and 2013.

Land use type	SOC (g kg^{-1}) 1990	SOC (g kg^{-1}) 2013
Cropland	10.3a	9.5a
Conversion	8.1a	5.1b
Forest	7.5a	10.0b
Wetlands	9.3a	10.7b
Rangelands	13.2a	13.7a

Means within rows followed by the same letter are not significantly different ($p<0.05$)

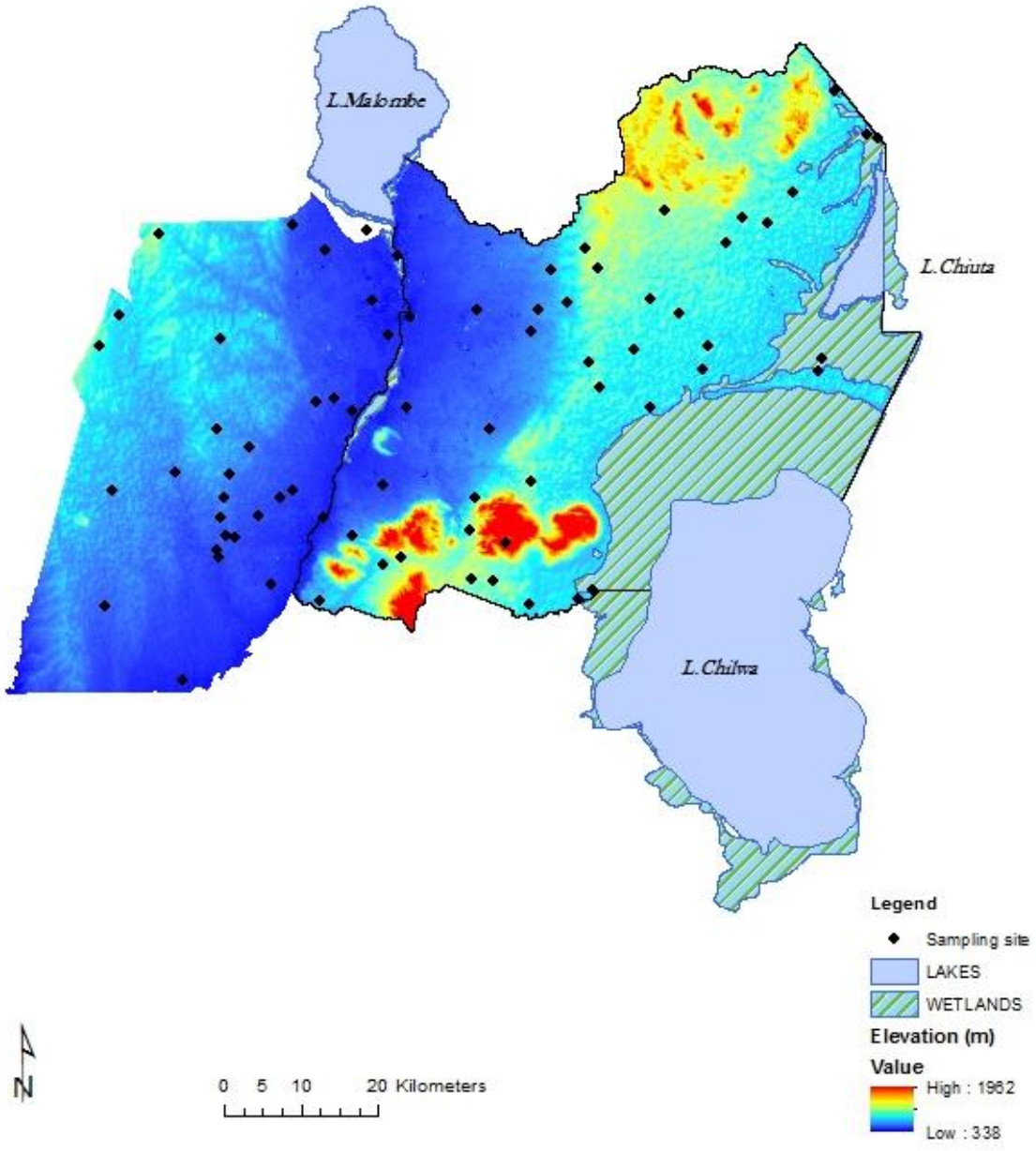

Figure 4.1. Map of Machinga District showing the elevation and location of sampling sites.

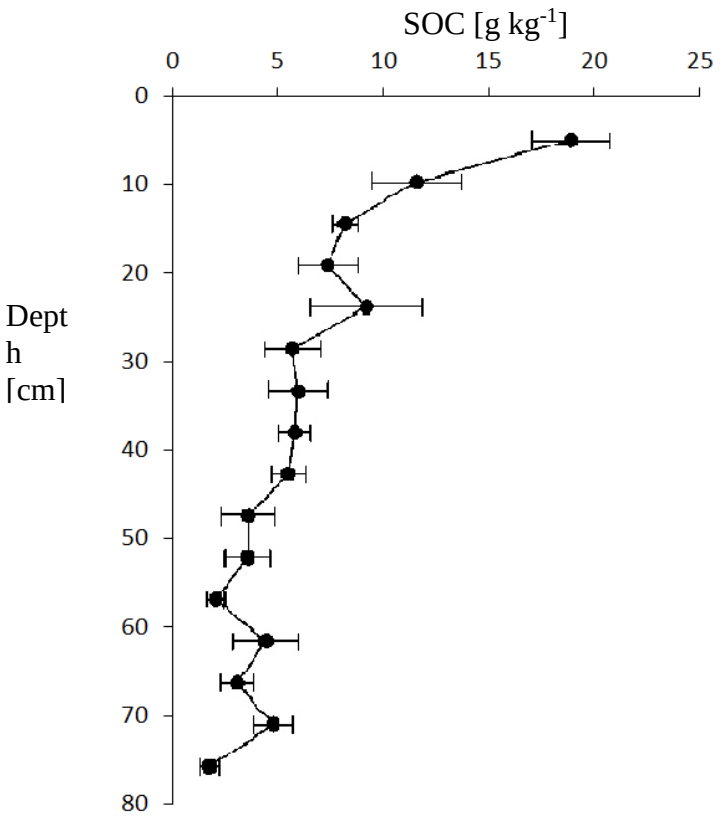

Figure 4.2. Vertical distribution of Soil Organic Carbon across sampling sites in Machinga District, Southern Malawi**.**

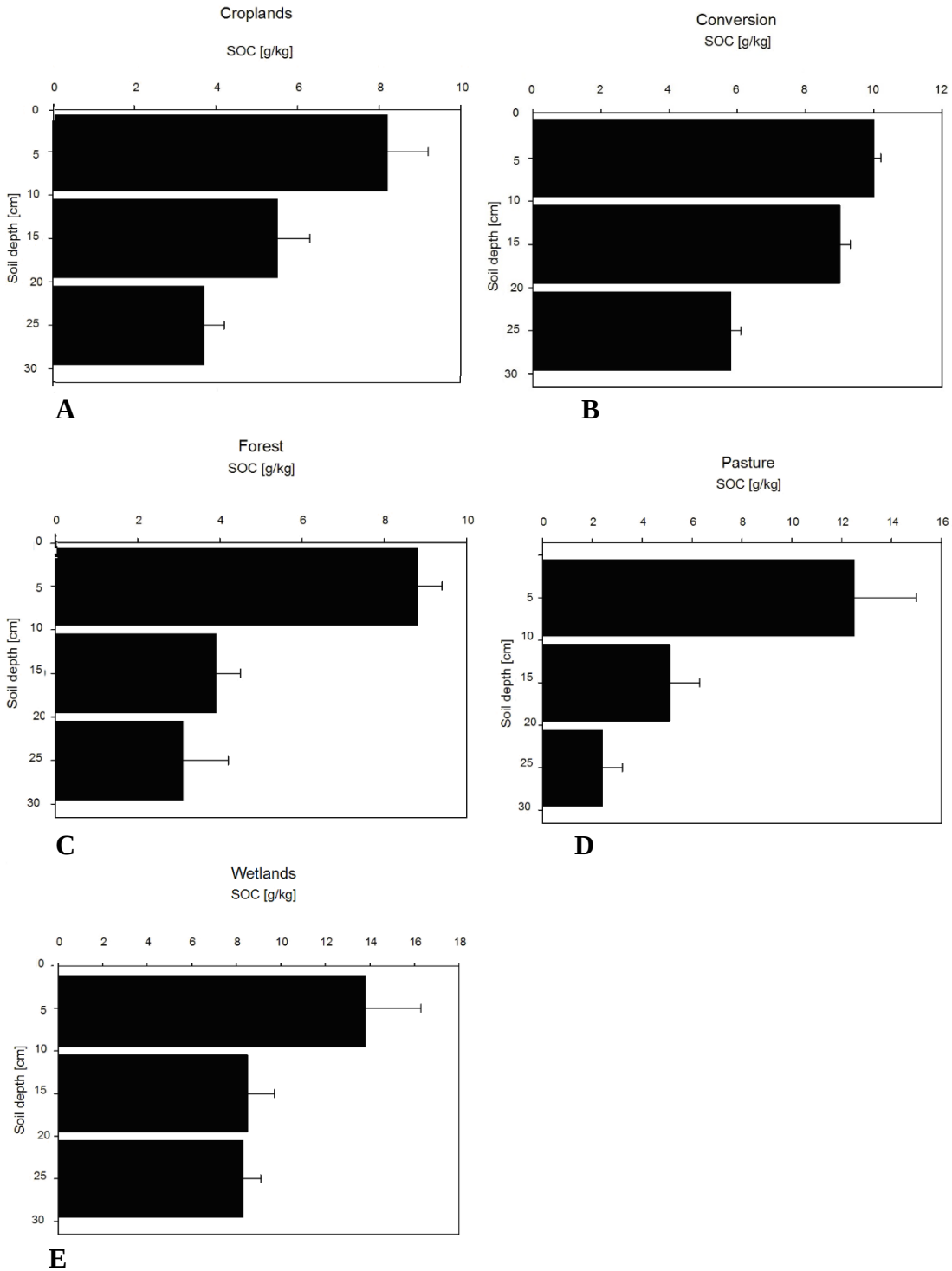

Figure 4.3. Vertical distribution of SOC by land use type in Machinga (0-30cm depth).

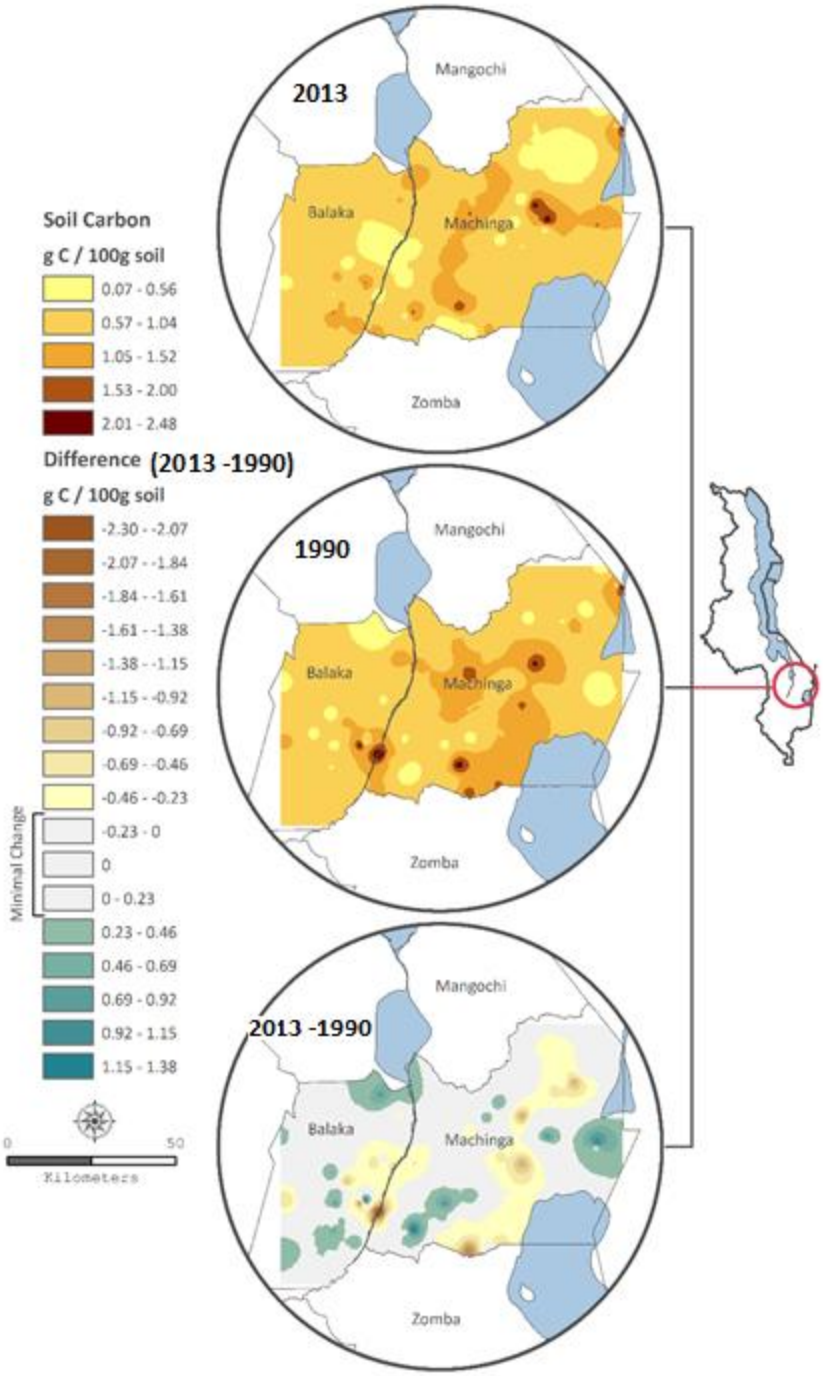

Figure 4.4. Changes in spatial distribution of Soil Organic Carbon in Machinga District, Southern Malawi after two decades

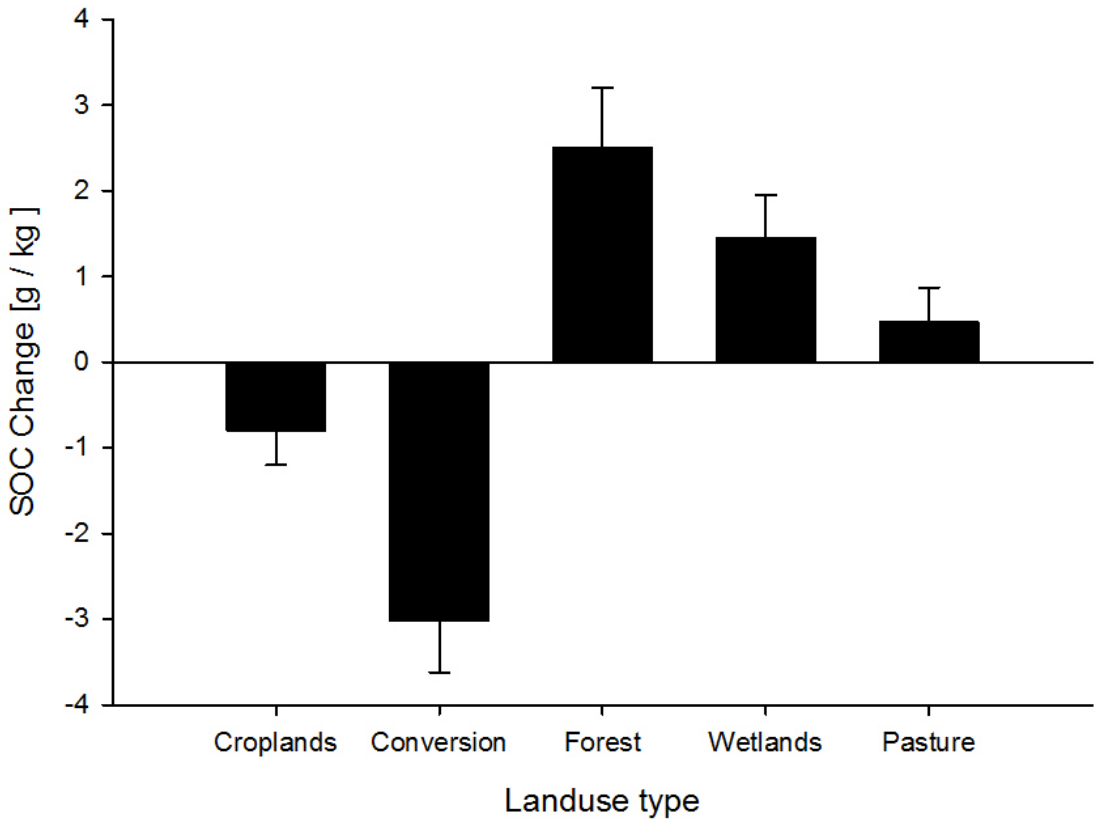

Figure 4.5. Changes in Soil Organic Carbon status across different land use types in Machinga District, Southern Malawi after 2 decades.

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