USING MICROBIAL INDICATORS

TO ASSESS SOIL ECOSYSTEM

RESTORATION

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure   Page

CHAPTER I

ABSTRACT

Soil microbial communities play a crucial role in maintaining ecosystem health and
sustainable integrity. The objective of this study was to determine the progress of
ecosystem recovery in soils under a federal conservation reserve program (CRP) by
examining several key microbial community-related soil health indicators. Three adjacent
soil ecosystems were evaluated, including conventional crop production, undisturbed
(>20 years post tillage), and marginal land that was formally cultivated and has been
under CRP for five years. The latter two ecosystems contained mixed perennial
vegetation, whereas the former was cultivated with winter wheat, *Triticum aestivum* L for
at least 20 years. Soils were taken from these three ecosystems at two different locations
twice a year for two consecutive years. Five replicate samples were taken from each
ecosystem at each location during each sampling event. Soils were evaluated for water-
stable aggregation and composition and structure of the soil microbial communities using
fatty acid methyl ester analysis. Roots isolated from soils were evaluated for arbuscular
mycorrhizal intraradical colonization.

Results show that AMF abundance was high in CRP plots compared to both Wheat and
Native systems. Microbial indicators suggest that the level of disturbance and nutritional
stress remained significantly higher in CRP than Native plots during the 1st and 3rd

samplings, indicating less resistance against heat or drought stress. Total microbial abundance and geometric mean diameter, which were highly correlated with each other, were found to be significantly lower in CRP than Native systems. Soil organic carbon and total nitrogen in CRP was not significantly different from the wheat fields, suggesting a much greater amount of time would be needed to reach pre-cultivation levels.

Because of significant differences between the two Native plots at different locations, using this study's selected indicators for soil ecosystem recovery, we were able to ascertain based on a combined value for measured indicators that the CRP at one location had recovered approximately 50% compared to the Native system, but that the other only recovered about 10% after 5-6 years in the program. The Native plot at the former location was possibly more degraded before being abandoned, or was less disturbed than the latter. Because a typical contract with the CRP typically lasts 10-15 years, more time may be required for full recovery of the soil properties measured in this study than what is currently allotted.

CHAPTER II

INTRODUCTION

On both a global scale and a national scale, resource managers as well as scientists and policy makers are grappling with problems that are uniquely tied together: the growing concentration of greenhouse gasses in the atmosphere and the degradation of farmland through poor management practices, as well as the loss of topsoil in general through various practices such as forest clearing and some conventional agricultural practices. With the exponential increase in the global human population, there is an accompanying pressure to expand farmable land into areas once considered marginal or which had previously been covered by forest or undisturbed habitats in order to produce enough to support the growing demand for food, clothing, fuel, and other goods. Pressure to expand into new territory is partly due to the mismanagement of existing farmable land with the use of unsustainable practices to a point that it is no longer favorable for crop production, especially when subjected to drought, in which case the cropland is abandoned. Given the current trend of unpredictable and extreme weather, it is of even greater concern to protect what arable land is currently available. Western Oklahoma and much of the high plains area of the United States during the dust bowl of the 1930's is a classic example of how mismanagement can have a significant, lasting impact on soil ecosystems (Hornbeck 2009).

Changes in land use that convert native vegetation to agriculture typically involve an overall decrease in vegetation and are known to have made significant and ongoing contributions to atmospheric greenhouse gas accumulation and loss of soil organic matter and topsoil. The reduction in vegetation coupled with standard farming practices in crop production deplete the soil of organic carbon and other stable nutrients, negatively affecting both the soil stability and tilth, as well as contributing to the net accumulation of greenhouse gases known to influence climate change.

Several successful strategies have been implemented in agriculture to mitigate greenhouse gas production and thwart SOM loss and soil erosion. Such strategies include conservation practices (e.g., ridge tillage, no tillage), addition of organic amendments in crop production, cropping intensification, switching to biomass crops (e.g., switchgrass), as well as converting to perennial vegetation (establishment of pasture or forest, Conservation Reserve Program) (Post et al, 2004). For the latter, surveys have shown that converting agricultural systems to established grasslands can result in an average rate of soil carbon accumulation ranging from 10 to 40 $g\text{-}m^2\text{-}y$ with the highest rates occurring in humid regions (Post and Kwon 2000). According to a 2007 study, it was estimated that grass cover planted under the Conservation Reserve Program (CRP) helped sequester more than 50 million metric tons of carbon dioxide, with the effect of the conservation practices on soil loss translating to an average nationwide net increase in total organic carbon of 0.7 metric tons per acre annually. It also estimated CRP reduced the amount of sediment runoff by 207 metric tons in 2007. (Food and Agricultural Policy Research 2007).

The Conservation Reserve Program, formally handled by the U.S. Department of Agriculture, was partially established in a nascent form with the Agriculture act of 1954, and officially established with the passing of the Farm Bill of 1985. Subsequent Farm Bills have expanded the program so that today CRP covers more than 30 million acres in the United States. As of April 2010, 861,286 acres in Oklahoma are enrolled in the conservation program (<3% of total).

The primary focus of the CRP is to counteract erosion on high-risk cropland by removing them from agricultural production and establishing long-term vegetation such as grasses and trees, and restoring riparian buffers and wetlands. Its purpose is also to improve water quality and enhance wildlife habitats. The program is set up so that landowners receive rental payments that are determined by the level of productivity of the area's soils and the average dry land crop rent or cash equivalent. In return, landowners establish approved vegetation cover, with the program providing up to 50% of the cost. As an additional financial incentive for the landowner, the program will provide for part of the cost of upkeep of enrolled land. These contracts between landowners and the USDA last between 10-15 years. However, due to federal budget constraints, only a fixed amount of land can be brought into the program, so gaining a contract can be competitive and current contract may not necessarily be renewed.

The CRP has proven to be successful in its objectives, and has seen a reduction in nitrogen, phosphorous, and sediment leaving the field via runoff. The Food and Agricultural Policy Research estimated that 278 million pounds less N and 59 million pounds less P left fields in 2007 due to CRP, 95 and 86% reductions, respectively (Food and Agricultural Policy Research 2007).

My study seeks to evaluate whether the CRP is successful in restoring the soil ecosystem of marginalized rangeland to pre- or near pre-cultivation status within the timeframe allotted for a typical contract. Former cropland that is currently under the CRP will be compared side-by-side to undisturbed (20+ years) grassland and a field under a wheat-fallow system that has been conventionally farmed for at least 20 years. There have been few studies focusing on the efficacy of soil ecosystem restoration of the CRP, or estimating the time estimated to recover to pre-cultivation status. Microbial indicators, particularly those pertinent to the abundance of arbuscular mycorrhizal fungi will be employed to distinguish differences between soil ecosystems.

CHAPTER III

REVIEW OF LITERATURE

<u>Soil Quality and Health, and the Conservation Reserve Program</u>

The concept of soil quality and health is not easy to define; however, the concept needs some clarification to assess the efficacy of restoration strategies. Karlen et al. (1997) defined soil quality as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation." Kibblewhite et al. (2011) adds that within an agricultural system "soil health is dependent on the maintenance of four major functions: carbon transformations, nutrient cycles, soil structure maintenance, and the regulation of pests and diseases." Particularly in agricultural systems, it is of upmost importance to protect the vitality and sustainability of the soil that increases crop productivity, as topsoil loss by tillage, and water and wind erosion decrease agricultural productivity (Battison et al. 1987, Heckrath et al. 2005, Kosmas et al. 2001, Olson and Carmer 1990, Papernik et al. 2005 and Tsara et al. 2001). The loss of topsoil is an example of a critical problem many agricultural systems are facing that stems from favoring conventional cropping over maintaining soil health; topsoil is a critical component of the soil ecosystem that is easily lost, yet slow to be replaced or rebuilt. Because the soil is eroded by wind or water, soil that has been

regularly tilled is most vulnerable, particularly between plantings when the soil is bare and exposed. One study in particular showed the negative effects of topsoil loss were still apparent even after 20 years (Lindstrom et al. 1986). The first step to rebuilding or even protecting the soil from erosion is by maintaining the health of the soil, which includes practices that contribute to its stability.

 Soil organic matter is another critical component of soil that is also quickly depleted from conventional cultivation, and the timescale in regaining SOM through conservation practices is on the order of decades. Within the first several years of the cultivation of native lands, soil organic matter decreases dramatically until the ecosystem reaches a steady state that stabilizes the loss (Lal 2002). The loss of soil C from agricultural practices, primarily as a result of tillage, is due to accelerated C oxidation by increasing soil aeration and exposing soil organic matter to accessible microbial mineralization (Paustian 1997).

The conversion from native mixed-plant species diversity to a monocultural agricultural system has its own indirect effects on soil tilth and stability in that plant diversity and microbial diversity are positively correlated (Waldrop et al. 2006) and increased plant species diversity is likely to increase the composition and quality of organic matter, a determinant in soil aggregation (Bossuyt et al. 2001). A typical agricultural system receives little input of organic matter to the soil which is an important food for microorganisms. Microbes have a variety of benefits including the degradation of potential pollutants, controlling diseases, and binding soil particles into large aggregates.

Although it is difficult to assess how long it may take for CRP to restore damaged land to pre-cultivation status due to many contributing factors, some previous studies have sought to identify the efficacy of the program in soil ecosystem restoration. One study compared a native grassland to both conventional cropland and land that had been abandoned for approximately 50 years found that the abandoned fields were not significantly different from native fields with respect to microbial biomass, potentially mineralizable N, or respirable C, suggesting that 50 years would be a safe estimation for recovery of active soil organic matter and nutrient availability (Burke et al. 1995). This study also suggested that plant dynamics were a major determination in recovery. Munson et al. (2012) studied SOC and TN dynamics in CRP fields seeded with either native or non-native plant species after 18 years of enrollment and found that fields seeded with native perennial grasses had 60% of the total SOC and 67% of the total soil N in undisturbed shortgrass steppe. Fields that were seeded with non-native perennial grasses had recovered less than those seeded with native vegetation.

Aggregate Stability

Well-structured soil is an important quality of sustainable soil ecosystems due to its ability to mediate important functions such as moisture retention, root development and retaining and protecting carbon and nitrogenous compounds from decomposition. One of the most beneficial features of well-aggregated soil is its resistance to erosion by wind and water. Aggregate formation and stabilization are affected by several factors, including the quantity and type of organic matter, iron- and aluminum oxides, and the clay content of the soil, with the primary stabilizing agent being organic materials (Lynch and Bragg, 1985).

It has been shown that arbuscular mycorrhizal fungi, a dominant microorganism in prairie ecosystems, have a variety of influences on the formation of aggregates on several scales (Rillig and Mummey 2006) in addition to their well-documented role in plant nutrition (Smith and Read 1997). At the ecosystem scale, AM fungi play a critical role in the formation of soil structure (Miller and Jastrow 2000) and in regulating C flux from plants to soil (Zhu and Miller 2003). They can consume up to 20% of plant C (Jacobsen and Rosendahl 1990; Watkins et al. 1996) and are often the largest contributor to soil microbial biomass (Miller, Reinhardt et al. 1995). Estimates indicate fungal structure and storage compounds are incorporated into the soil matrix with relatively long residence times, and contribute to a slower turnover pool of soil organic C (Olsson and Johnson 2005).

Arbuscular mycorrhizal fungi directly contribute to soil aggregation through the physical entanglement of soil particles with external hyphae (Tisdall and Oades 1982). Additionally, AM hyphae have been found to produce a stable hydrophobic glycoprotein, glomalin, which acts as a long-term binding agent (Wright and Upadhyaya 1996, Wright and Upadhyaya 1998, Rillig et al. 2005). Several recent studies have shown high linear correlations between glomalin or glomalin-related soil proteins, in aggregates and aggregate stability (Wright and Anderson 2000, Rillig et al. 2002, Rillig et al. 2003). As soil aggregation is thought to protect C rich detritus from microbial degradation, an increase in aggregate stability could prove to be important in increased sequestration of C (Jastrow 1987, Six et al. 1998, Miller and Jastrow 2000, Six et al. 2000)

Because AM fungi are dominant terrestrial microorganisms in undisturbed prairie ecosystems, and the presence of AMF is strongly correlated with greater water-stable soil

aggregation and carbon and nitrogen storage in the soil (Wilson et al. 2009), their presence could be indicative of a favorable recovery process in such soil conservation strategies such as the CRP.

<u>Relationships between soil organic matter and microbial biomass</u>

Patterns of soil C storage and cycling are determined by the balance between organic matter inputs resulting from primary production (e.g. roots and root exudates) and losses due largely to microbial respiration processes. The microbial contribution to C cycling, in turn, is determined by microbial community structure and soil properties such as aggregate stability (Six et al. 2006). Soils with higher proportions of fungal biomass relative to bacterial biomass are associated with greater C storage and slower C turnover, although the involved mechanisms remain unclear (Thiet et al. 2006). The proportions of fungi and bacteria are responsive to soil disturbance and substrate quality, with fungal-dominated communities favored in minimally disturbed ecosystems and by low quality (high C:N ratio) substrates (Bardgett et al. 1996, Bossuyt et al. 2001). Natural grasslands represent classic examples of fungal-dominated decompositional pathways driven by high C:N ratios of the dominant flora, with saprophytic fungi accounting for as much as three-fourths of total microbial respiration in tallgrass prairie (Rice et al. 1998).

CHAPTER IV

METHODOLOGY

Experimental Design

Soils were taken from two locations (Location 1: N 36.96076, W 97.62963; Location 2: N 36.98516, W 97.60532) near Medford, Oklahoma, USA in the north-central part of Oklahoma. The native/undisturbed vegetation is typical of the southern mixed prairie, dominated by perennial grasses with variable statures. The soils are classified as Kirkland silt loam (fine, mixed, superactive, thermic udertic paleustoll). Each location contained three different adjacent management practices: Native (undisturbed) prairie, known to be undisturbed for at least 20 years (Native), Conservation Reserve Program (CRP), and winter wheat, *Triticum aestivum L.* (Wheat) which had been under continuous cultivation for at least 20 years. The CRP land was seeded with USDA-NRCS approved seed mixture to establish perennial vegetation (Table 1).

Soil Collection

Soil samples were taken June 10th-11th and July 29th-30th in 2009 and June 2nd-3rd and August 4th-5th in 2010 for a total of four sampling events. Each year, the first sampling took place during peak plant production in late spring/early summer prior to excessive

Table 1 Percent composition of seed mixture applied to land under Conservation Reserve Program at locations 1 and 2 near Medford, Oklahoma (Courtesy of Johnston Seed Co.)

| | Location 1 | Location 2 |
Seed type	-------% of total seed mixture-------	
Little bluestem	20	22
Big bluestem	15	7
Indiangrass	10	5
Switchgrass	10	3
Sideoats grama	20	22
Blue grama	15	11
Illinois bundleflower	4	6
Partridge pea	4	1
Maximillian sunflower	2	0
Buffalograss	0	24

drought and heat stresses. According to historical rainfall data (Mesonet website), June is the wettest month in this region. The second sampling was performed during late summer in the midst of peak average air temperatures and decreased rainfall, with June having the greatest amount of rainfall (Figures 1 and 2). We hypothesize that plant and microbes in the tested ecosystems would encounter heat/drought stresses and their physiological responses are detectable if the right indicators were selected. Although historical data showed these trends, the actual sampling dates had somewhat different trends; the "spring" (early summer) samplings were somewhat dryer than the "summer" (late summer), receiving significantly less rainfall in the week preceding, although the air temperature was slightly cooler. The final sampling event had seen the highest quantity of rainfall within the previous week (Table 2).

Within each system at each location, five replicate plots of $1x1m^2$ squares were randomly placed. Within each square, pH and soil temperature was measured; all vegetation was removed (except in the Wheat treatment) and collected, and five soil cores of the top 5 cm surface soil were taken to form a composite soil sample. Soils were stored and transported on ice to the lab and immediately placed in a 4°C cold room until processing and analysis were performed. Soils were first run through an 8mm sieve to remove debris and solid fragments; roots were also removed and reserved. A portion of the soil was set aside and air-dried for aggregate stability and chemical analysis, while another portion was lyophilized for FAME extraction and ground with a mortar and pestle. Any remaining soil was returned to 4° cold storage. Samples were finely ground for the analysis of total elemental nutrients and FAME analysis.
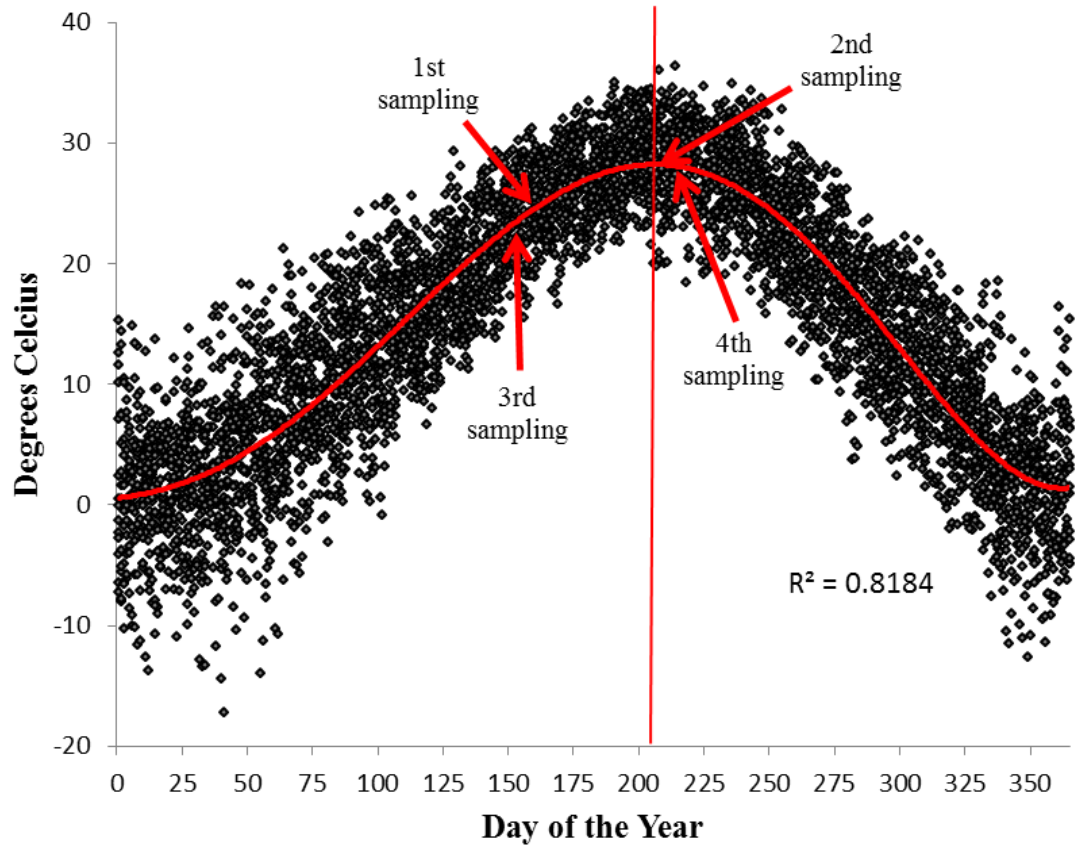
Figure 1 Daily average air temperature (°C) from March 1997-December 2011 measured near Medford, Oklahoma (www.mesonet.org). Sustained maximum air temperature typically occurs around the end of July to the beginning of August, DOY (day of year) 205-215.
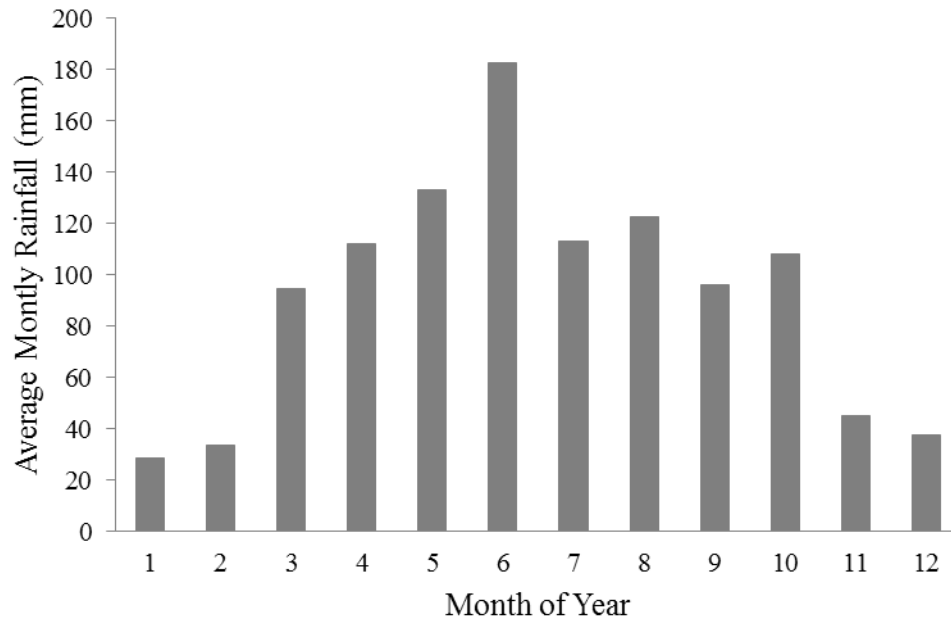
Figure 2 Average monthly rainfall (mm) from January 1995-December 2010 measured near Medford, Oklahoma (www.mesonet.org)

Sampling period	Total rainfall (mm)	Average air temperature (°C)
1	12.70	23.5
2	17.02	25.2
3	0.51	25.2
4	68.33	30.5

Soil nutrient analysis

Soil moisture content was determined gravimetrically after drying at 105°C for 48h. Soil texture was determined using a composite sample consisting of 3 reps within each system at each location and determined by the hydrometer method (Gee and Or 2002). Samples from the 4th (final) sampling event were used for the analysis of pH, KCl extractable inorganic N (NO_3-N and NH_3-N) and Mehlich-III extractable phosphorous. Two samples from each system at each location were chosen from the 4th sampling event to test for total nitrogen (TN) and soil organic carbon (OC). Phosphorous was analyzed using a Spectro ICP (Mehlich III extraction) (Gavlak et al. 2003). Extracted inorganic N was analyzed using a flow-injection analyzer (Gavlak et al. 2003), SOC and total nitrogen were tested with a Leco TruSpec combustion analyzer (Bremner 1996, Nelson and Sommers 1996).

Aggregate stability by wet sieve analysis

After field moist soil was passed through an 8 mm sieve, a portion was thinly spread out and left to air dry for 48h. Upon drying, soil was placed on a 4 mm sieve and gently shaken to select dry aggregates greater than 4 mm to have a standardized aggregate size prior to wet-sieving. 50 g of this soil was placed on the top sieve of a wet-sieving apparatus similar to that described by Yoder (1936) that consists of 5 stacked sieves (4mm, 2mm, 1mm, 0.5mm, and 0.25mm) with the capability of running two samples simultaneously. Each set of stacked sieves is wet-sieved in a 5-gallon bucket filled with enough distilled water to completely submerge the soil on the down stroke of the apparatus. Soil is pre-wetted for 10 minutes by slowly lowering the sieves into the water until the soil is just covered by the water on the upstroke of the machine. Following 10

minutes of pre-wetting, soils are then wet-sieved for an additional 10 minutes at 30

rotations a minute. Soils from each sieve are then transferred to individual beakers by

washing with distilled water. Beakers are placed in an oven to dry and then weighed. The

geometric mean diameter (GMD) was calculated (Mazurak 1950).

$$GMD = \exp[\Sigma_{i-1}^n w_i \log \bar{x}_i /\Sigma_{i-1}^n w_i]$$

$\bar{x}_i = mean\ diameter$

$w_i = weight\ of\ aggregates\ in\ a\ size\ class$

$\Sigma_{i=1}^n w_i = total\ weight\ of\ sample$

Soil microbial community analysis

The microbial community was evaluated by extracting ester-linked lipids from soil and

by extracting ester-linked lipids and analyzing fatty acid methyl esters (EL-FAME), a

method described by Schutter and Dick (2000) with some modifications. Briefly, the

method is done with four steps: 1) saponification of fatty acids by incubating 3 g of

lyophilized soil in 7.5mL 0.2 M KOH in MeOH at 37° for 60 min; 2) methylation in 1.5

mL 1.0 M acetic acid, and 3) organic extraction in 3ml 1:1 hexane:methyl-tert-butyl 4)

concentration under N_2 and redissolved in a clean vial in 100μl 1:1 hexane:MTBE.

FAMEs were analyzed in a 6890 GC Series II (Hewlett Packard, Wilmington, DE, USA)

equipped with a flame ionization detector and a fused silica capillary column (25 m x 02

mm) with H_2 as the carrier gas. The program temperature was ramped from 170°C to

250°C at 5°C min-1. FAMEs were identified and quantified by comparison of retention

times and peak areas to components of standards. FAME concentrations were calculated

by comparing peak areas to an analytical standard (19:0, Sigma Chemical Co., St. Louis, MO) calibration curve. The fatty acids are described by the number of C atoms, followed by a colon, the double bonds and then by the position of the first double bond from the methyl (ω) end of the molecule. Cis isomers are indicated by c, and branched fatty acids are indicated by the prefixes i and a for iso and anteiso, respectively. Other notations are Me for methyl, OH for hydroxyl, and cy for cyclopropane. FAME data is reported in percent of the total mole fraction, which can be interpreted as relative abundance of the total detected microbial community. We primarily focused on 4 markers for this study: 1) 16:1w5c for arbuscular mycorrhizal fungi (Graham et al 1995, Jansa et al 1999, Olsson, 1999) 2) the ratio indicating nutritional stress, total saturated/total monounsaturated fatty acid ratios ($12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0$)/($14:1\omega5c + 15:1\omega6c + 16:1\omega7c + 16:1\omega5c + 17:1\omega9c + 18:1\omega9c + 18:1\omega7c$) (Guckert et al. 1986, Zelles et al. 1994), 3) The ratio indicating disturbance, cyclopropyl fatty acids/monoenoic precursors (19:0cy/18:1w7c), (Kieft et al. 1997 and Fierer et al. 2003), 4) fungal markers 18:1w9c and 18:1w7c (Wright, 1983 and Frostegård et al. 1993) and 5) total extracted FAMEs.

Assessment of AM fungal root colonization

Roots were collected and stained with some adaptation from Philips and Hayman (1970). Briefly, fresh roots were retained from collected soils. A portion of the roots from each sample were placed in test tubes, thoroughly washed with distilled water and oven-dried. Roots were then soaked in 10% KOH at 90 °C for 1 hour. This step was repeated until roots no longer appear dark in color. Next, KOH was decanted from the test tubes and roots were acidified with diluted HCl for several minutes. Then, HCl was removed, and roots were stained by adding 0.05% Trypan Blue in lacto-glycerol for 10 minutes at 110

°C. Excess stain was removed from the tubes and roots were stored at room temperature in 33% lactic acid until further analysis.

Roots were scored for intramatrical AM colonization using the magnified gridline intersect method as described by McGonigle et al (1995). The method employs the use of a compound light microscope at 200x magnification to measure the percent root length colonized by AM fungi (total colonization).

Statistical methods

FAME and aggregate data was log transformed to conform to homogeneity of variances prior to analysis and is presented as actual values. Significant differences among treatments, seasons, and locations were determined using three-way analysis of variance (ANOVA). Comparison of means was conducted according to the least significant difference test (LSD, $p \leq 0.05$) using the general linear model procedure of the Statistical Analysis System (SAS, 1999).

CHAPTER V

RESULTS

Soil nutrient analysis

Soils from CRP and Wheat treatments were not significantly different in organic carbon

or total nitrogen contents. Both CRP and Wheat systems contained less than soils from

the Native system. Soil texture had no detectable differences across systems evaluated

(Table 3). Soil pH values were significantly different in different systems and the trend

was consistent at both locations (Table 4) with Wheat systems being the most acidic,

Native nearly neutral, and CRP in between. Extractable inorganic N (NO_3-N and NH_4-N)

levels varied by systems as well as by location. At location 1, NO_3-N and NH_4-N

concentrations were not significantly different across different systems. However, in soils

at location 2, concentrations were significantly higher in Wheat than CRP or Native.

Considerable variations in extractable inorganic N levels, especially for NH_4-N, were

observed between the tested two locations, with higher levels at location 1. The trends

detected for extractable P levels were different from extractable inorganic N levels; at

location 1, the lowest extractable P was detected in Wheat; whereas at location 2, the

lowest was in Native.

Table 2 Contents of organic carbon (OC), total nitrogen (TN), and texture in soils of the three systems studied. Data presented are averages of all soil samples within the same system across two locations. Different letters within each column indicate means are statistically different (p<0.05; n=3 for each system) according to least significant difference test.

System	OC	TN	Sand	Silt	Clay
	------ % ------		------- % --------		
Wheat	1.17^b	0.12^b	17.5^a	58.7^a	23.8^a
CRP	1.47^b	0.13^b	16.9^a	56.3^a	27.0^a
Native	2.00^a	0.18^a	18.2^a	53.8^a	28.2^a

Table 3 pH, KCl-extractable NO_3-N and NH_4-N, and Mehlich-3 P for both locations within each treatment. Different letters within each column indicate means are statistically different (p<0.05; n=5) according to least significant difference test.

System/Location	pH	NO_3-N	NH_4-N	P
		----- mg kg^{-1} -----		
Wheat				
L1	4.9^d	23.1^{bc}	6.7^{ab}	30.8^{bc}
L2	4.9^d	76.7^a	10.6^a	71.1^a
CRP				
L1	5.9^c	7.1^c	7.6^{ab}	58.9^a
L2	5.9^c	36.2^b	5.7^b	22.0^c
Native				
L1	6.3^b	9.3^c	10.4^a	44.6^b
L2	6.7^a	9.3^c	5.2^b	5.6^d

Aggregate Stability by wet-sieve analysis

Aggregate stability was evaluated using samples obtained in 2010. The geometric mean diameter showed that there was some seasonal variability (Figure 3). Native fields were significantly higher in late Summer than in the Spring, with location 1 varying the most, changing from 0.75 to 2.5 from Spring to Summer. The GMD of the CRP fields was also lower in the Spring than in the Summer, but did not vary as much as Native. Native fields were significantly higher than both CRP and Wheat treatments in all cases with the exception of the Spring 2010 sampling at location 1, where there was no significant difference between Native and CRP. Wheat was much lower than all other treatments, regardless of sampling time or location, with GMD around 0.25mm.

However, there was a clear, consistent trend across treatments despite seasonal variability. Soil aggregate GMD and mean weight distribution (Figure 4) showed that while CRP and the Wheat fields were significantly different from each other, they were both considerably lower in terms of GMD, as well as content of larger water-stable aggregates (<4 mm and 2-4 mm) compared to the Native soils. The last column of figure 4 is the proportion of the 50 g sample that was too small for the smallest (0.25 mm) sieve, the fraction that was essentially "washed away" from the sample. This category showed an opposite trend where the Wheat soils had the highest proportion of sample (greater than 60%) too small to be detected from the analysis, and the Native soils with the least (in most cases less than 20%) and CRP roughly falling between the two at about 40-45% (Figure 4).
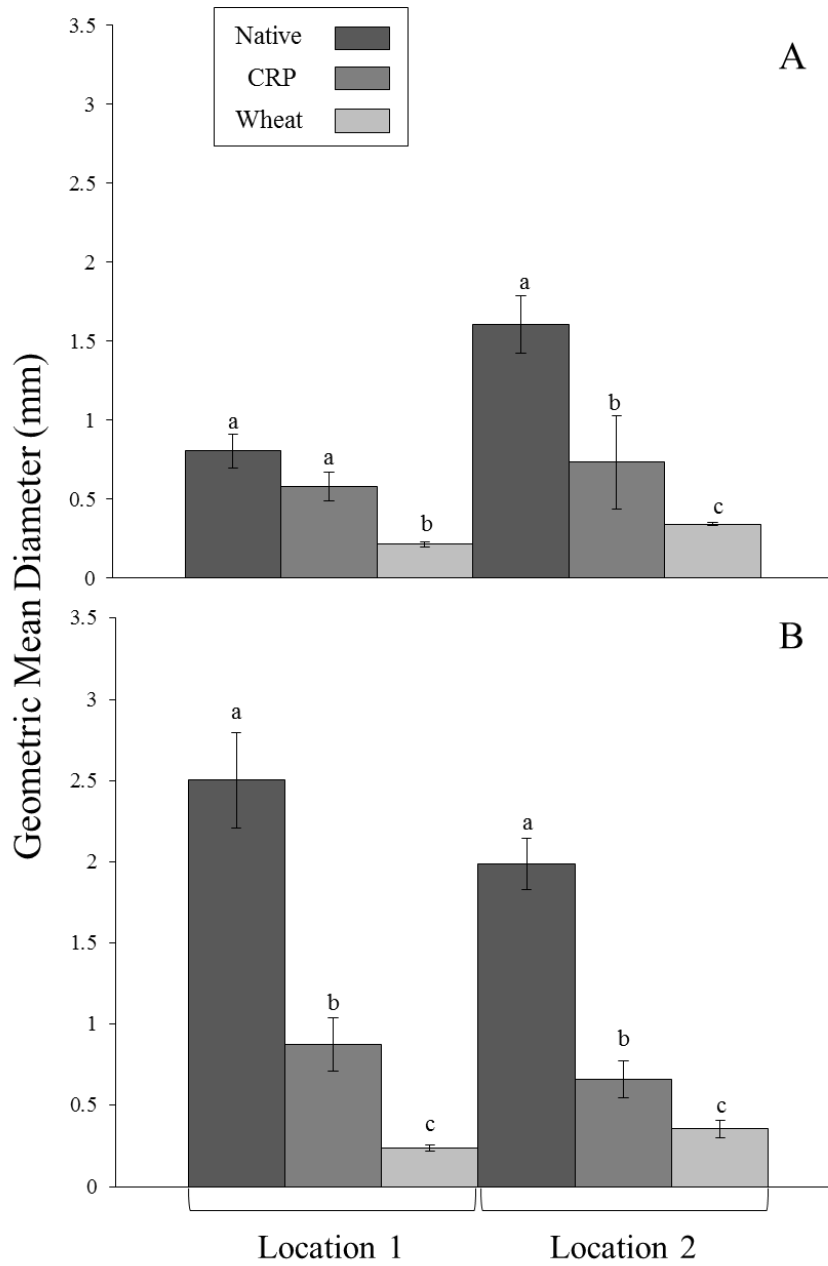
Figure 3 Combined mean values (+/- SE) of soil geometric mean diameter (GMD) of Native, CRP and Wheat treatments at each location (Loc 1 or 2) during A) Spring 2010 sampling and B) Summer 2010 sampling. Different letters within each column indicate means are statistically different (p<0.05; n=5) according to least significant difference test.
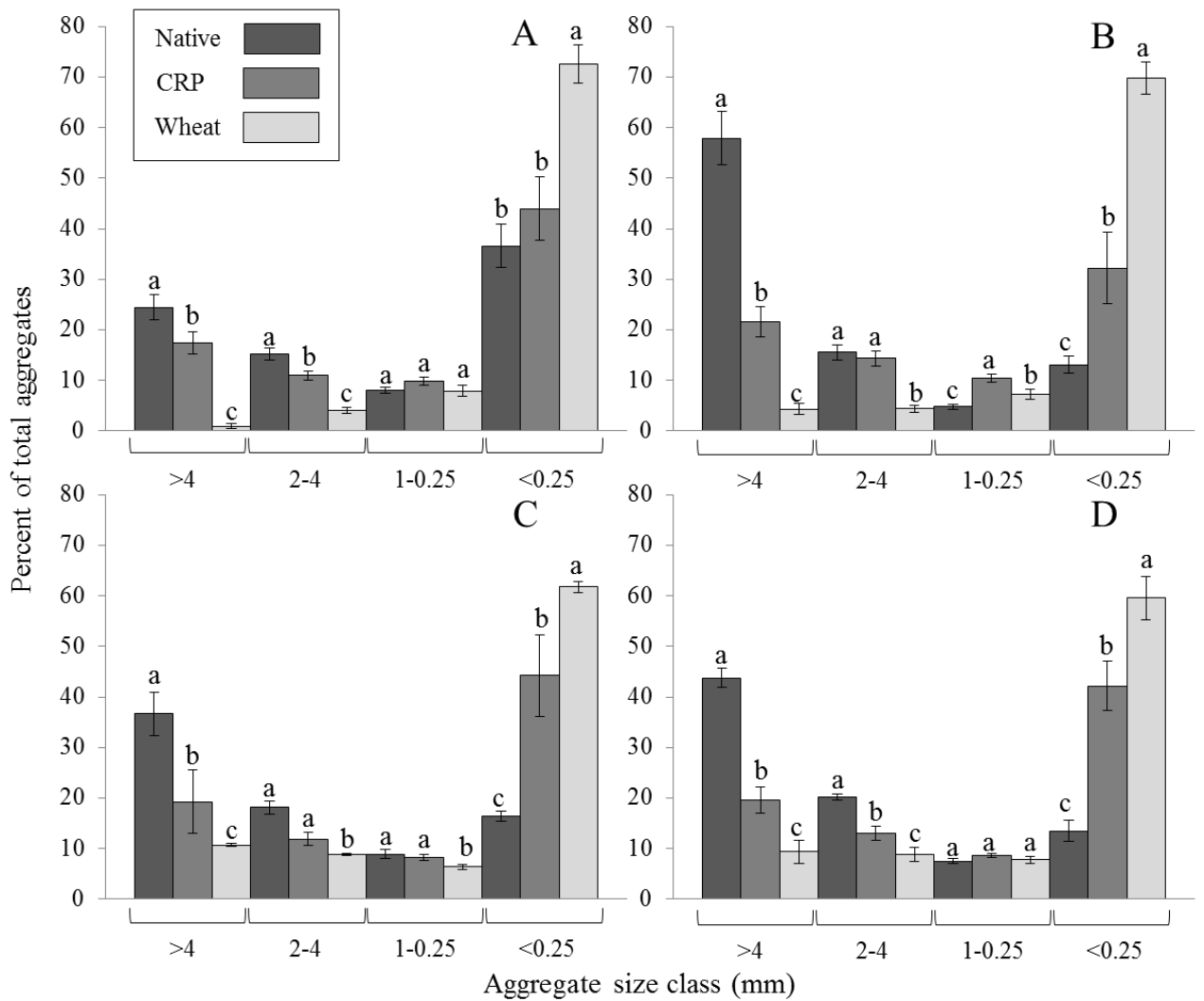
Figure 4 Weight distribution by sieve size +/- SE (percent of total 50g sample) of Native, CRP and Wheat treatments at each sampling: A) Spring 2010, Location 1 B) Spring 2010, Location 2 C) Summer 2010, Location 1 D) Summer 2010, Location 2. Different letters indicate means are statistically different according to least significant difference test (p<0.05; n=5).

Soil microbial community analysis

Fatty acid methyl ester (FAME) analysis was performed on all 120 soils from all four sampling events. This study focuses primarily on key FAME signatures that are relevant to interpreting the primary makeup of the soil microbial structure that might indicate soil health. The signature indicative of arbuscular mycorrhizal fungi, 16:1w5c, the fungal signatures 18:1w9c and 18:1w7c representing saprophytic fungi, and the ratios that represent the level of disturbance (19:0cy/18:1ω7c) within the community and nutritional stress (total saturated/total monounsaturated FAMEs), as well as the total abundance of extractable FAMEs were used to provide a general picture of the microbial community structure in regards to soil health.

The relative abundance of AMF (figure 5) did not show a consistent trend between the Native and CRP treatments at all four sampling times, however both were consistently higher than Wheat (<4%) in all cases. AMF abundance increased with each subsequent sampling in CRP, with the highest levels occurring in late summer 2010 (during the sampling with the most rainfall), often with greater abundance than the Native plots, particularly at location 1. The Native plot at location 2 had consistently greater AMF abundance than the Native plot at location 1.
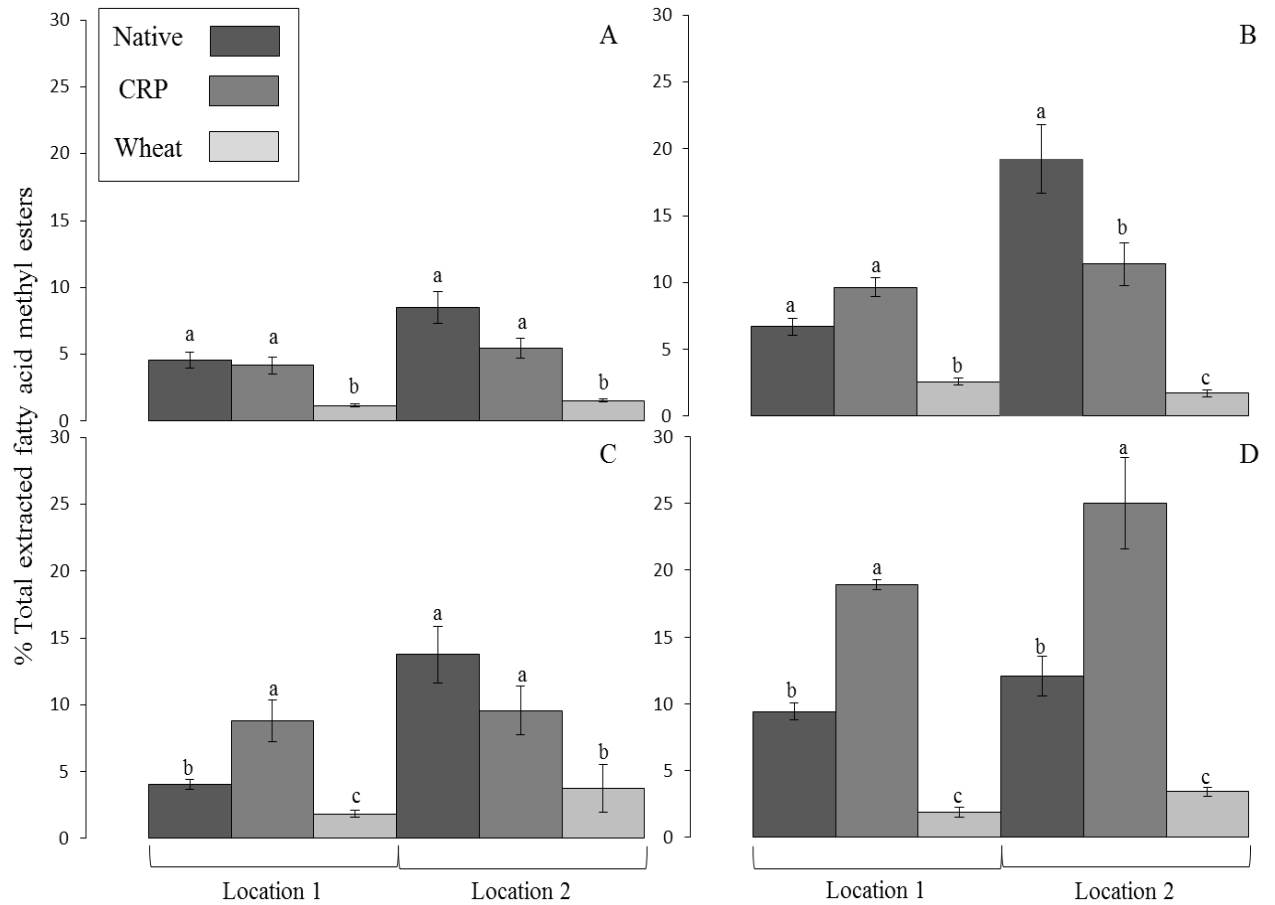
Figure 5 FAME profile 16:1ω5c, indicative of arbuscular mycorrhizal fungi presented as mean % total FAMEs +/- SE. A) Spring 2009 B) Summer 2009, C) Spring 2010, D) Summer 2010. Different letters indicate means are statistically different according to least significant difference test (p<0.05; n=5).

Saprophytic fungal abundance (figure 6) was fairly consistent with AMF abundance. The Native field at location 2 consistently had significantly greater fungal abundance than all other treatments at each sampling time. Relative to other treatments, saprophytic fungal abundance was lower than AMF in CRP plots, suggesting that AM fungi were a more dominant fungal group. For the majority of the sampling times, the highest levels were found in the Native fields (around 15-20%), with CRP and Wheat fields around 10-15% and frequently not significantly different from each other.

The FAME biomarker ratio indicative of level of disturbance had a distinct trend (Figure 7), with Wheat fields having a significantly higher level than the other two systems, generally between 1.5-2. Native and CRP treatments were not significantly different from each other, falling between 0.5-1, except for in the Spring of 2009 and 2010. During this time there was a detectable difference, with Native at the lowest, Wheat at the highest, and CRP falling between the two.
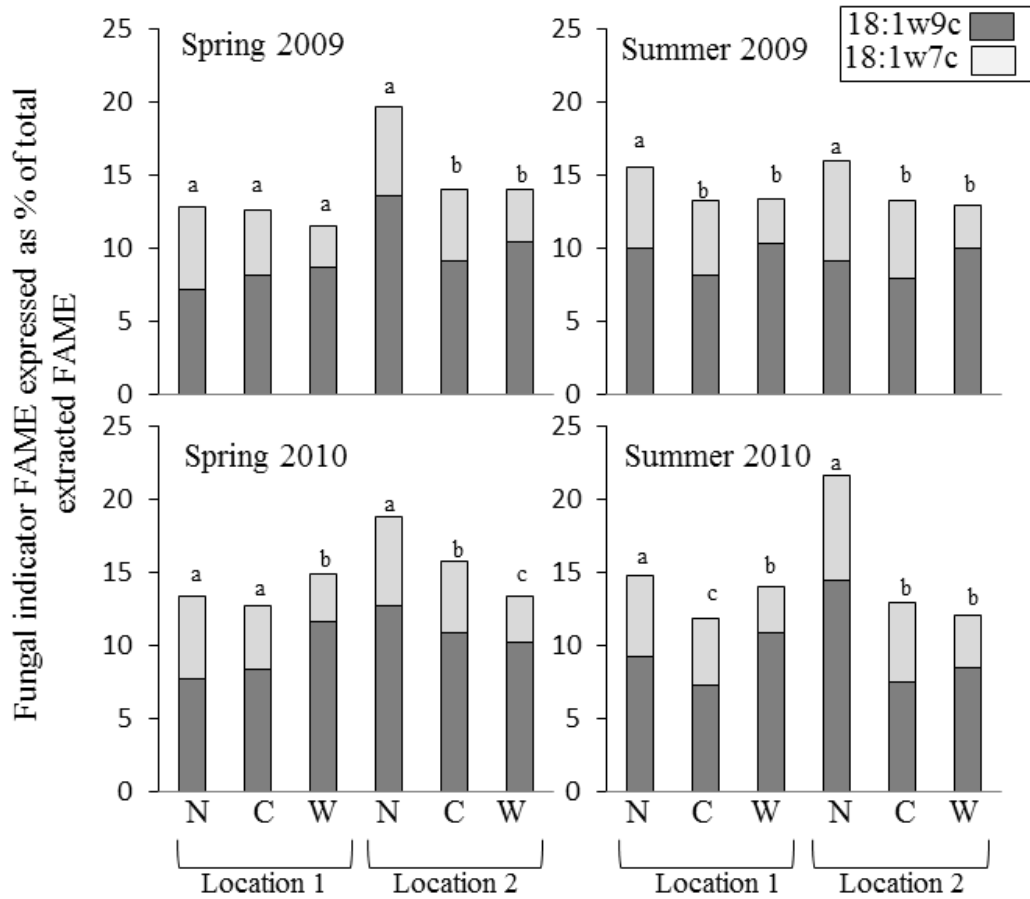
Figure 6 FAME fungal profiles 18:1w9c and 18:1w7c representing saprophytic fungi. N=Native, C=CRP, W=Wheat. Different letters indicate means are statistically different according to least significant difference test (p<0.05; n=5).
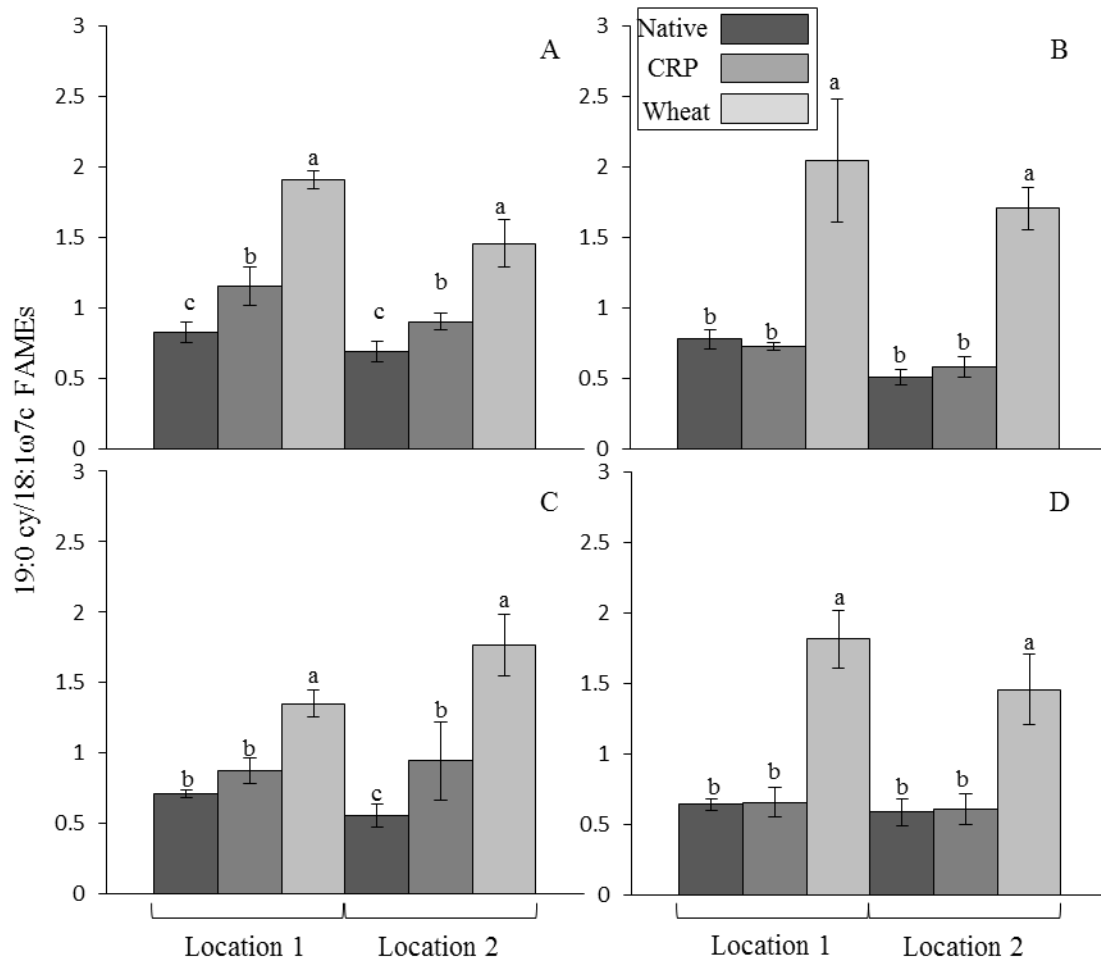
Figure 7 19:0cy/18:1ω7c FAMEs, indicative of level of disturbance +/- SE. A) Spring 2009 B) Summer 2009, C) Spring 2010, D) Summer 2010. Different letters indicate means are statistically different according to least significant difference test (p<0.05; n=5).

The ratio of the total saturated/total monounsaturated FAMEs, indicative of nutritional stress in the soil microbial community, followed a similar trend to that of the disturbance level ratio (Figure 8). The Wheat fields were consistently the highest at all sampling times, falling around 1.5-2.25. Levels in the Native and CRP treatments were somewhat variable; at location 2, during three out of four sampling periods, CRP was significantly higher than Native, whereas the remainder, and at location 1, CRP and Native were not significantly different from each other. In general, Native and CRP plots at location 1 had higher nutritional stress than the same treatment plots at location 2.

The total microbial abundance had an opposite trend of the nutritional stress ratio, with Wheat being consistently lower than Native, and with CRP either falling between the two, or at the same level as the Native fields (Figure 9). Wheat was fairly consistently around 40-45 nmol FAMEs g^{-1} soil, whereas the Native treatment was highly variable, reaching levels as high as 115 nmol g^{-1} soil (location 1, Spring 2009), and as low as 60 nmol g^{-1} soil (location 1, Summer 2009). CRP was slightly less variable, with levels generally around 40-60 nmol g^{-1} soil, except in the final sampling period (Summer 2010) when the extracted fatty acids were much higher across the board. Total extracted fatty acids in the CRP plots were also generally higher in late summer samplings vs. spring.
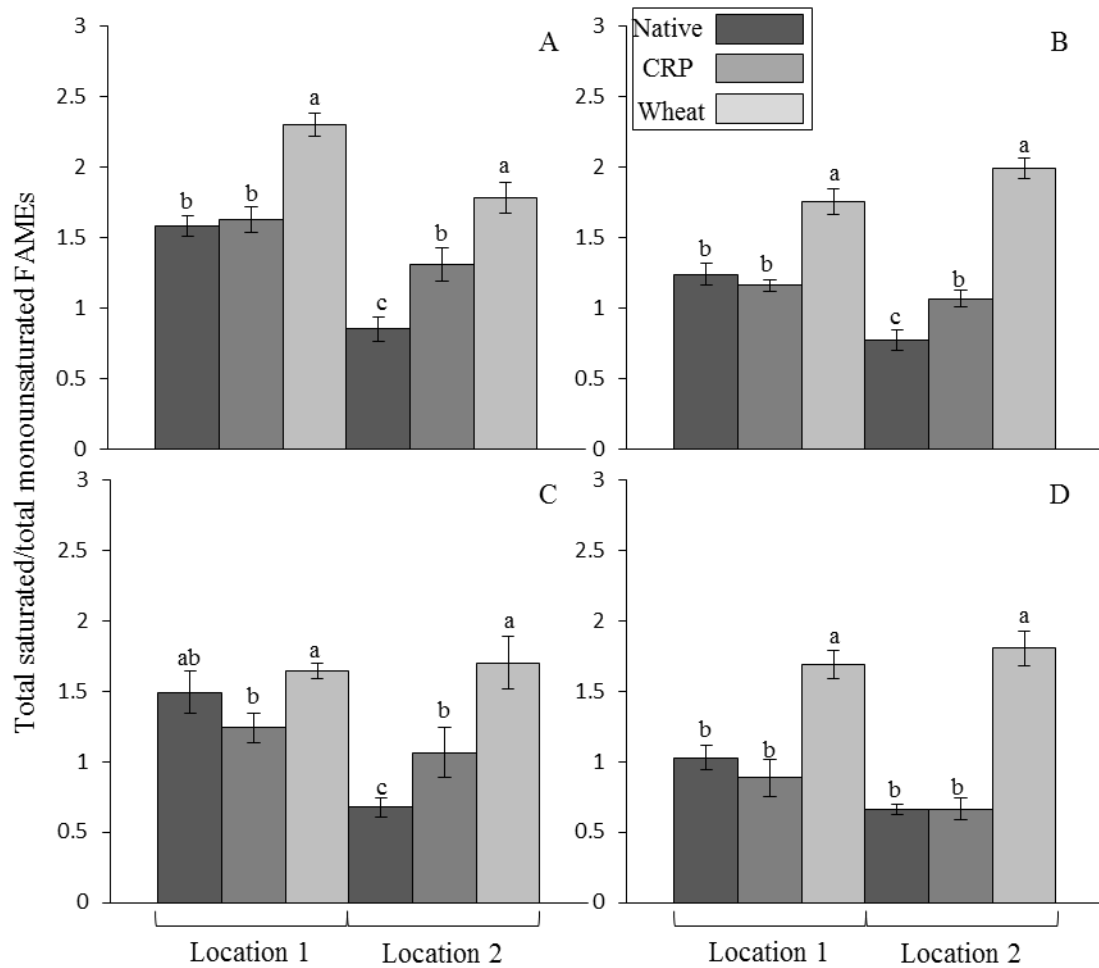
Figure 8 Ratio of the total saturated/total monounsaturated FAMEs, indicative of nutritional stress +/- SE. A) Spring 2009 B) Summer 2009, C) Spring 2010, D) Summer 2010. Different letters indicate means are statistically different according to least significant difference test ($p<0.05$; n=5).
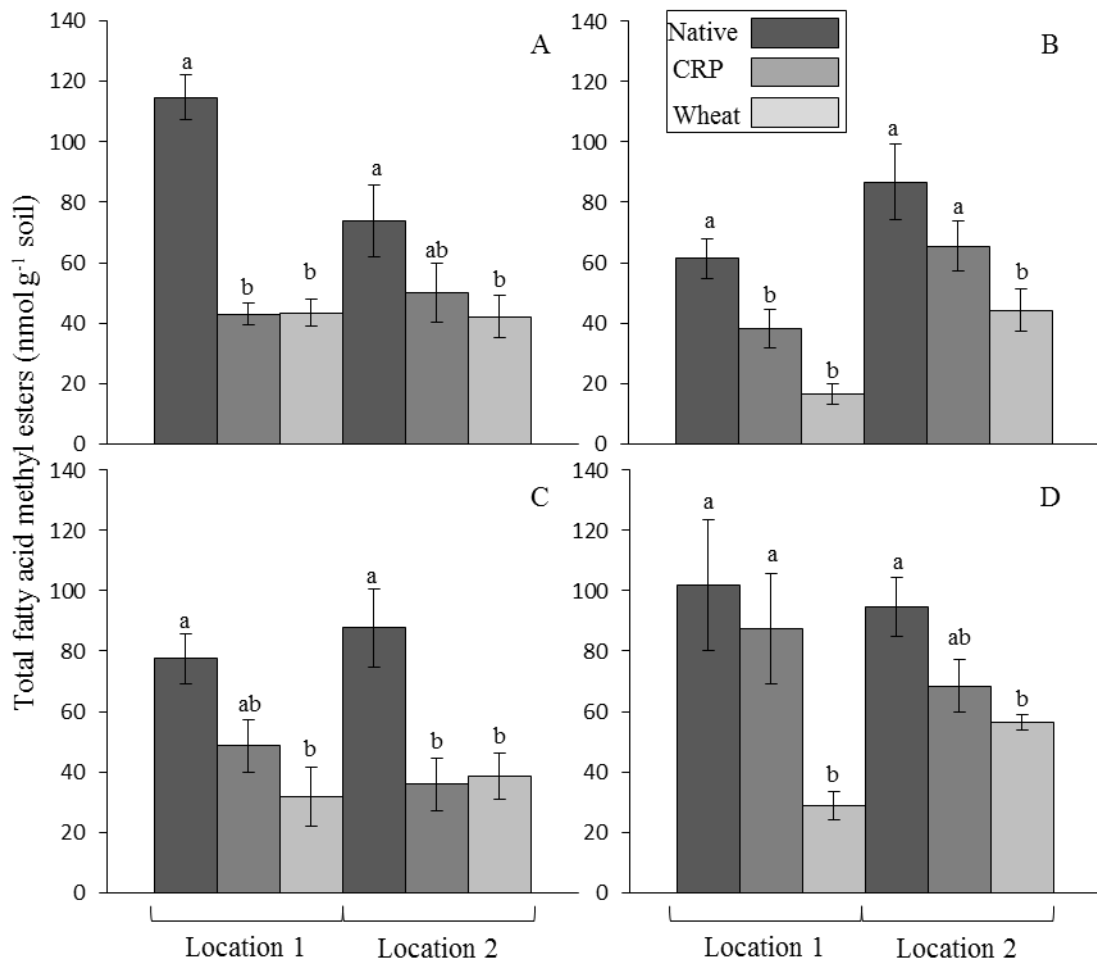
Figure 9 Total microbial abundance represented by the total extracted FAMEs (nmol g^{-1} soil) +/- SE. A) Spring 2009 B) Summer 2009, C) Spring 2010, D) Summer 2010. Different letters indicate means are statistically different according to least significant difference test ($p < 0.05$; n=5).

Assessment of AM fungal root colonization

Arbuscular mycorrhizal fungi intramatrical root colonization was measured for the last two samplings (early and late summer 2010) only (figure 11). The roots reserved from the first sampling period were destroyed in the process of staining, and the remaining roots in the soil were very young and all seemed to have similar AM fungal colonization after staining, so this set was probably not a true representation of AMF abundance in the soil at that time. The same could be said about the roots taken from the Wheat fields; one of the fields was fallow (no vegetation) at the time of sampling, and in the planted field, we were not able to sample directly under the plants, rather between the rows, so the roots that were present were quite small and difficult to analyze. Hence, the most meaningful comparisons would be between the Native and CRP plots in the Summer of 2010 (B from figure 11). The relative amount of AM fungal root colonization at location 2 is consistent with the AMF abundance ($16:1\omega5c$ FAMEs) but showed an opposite trend in the Native plots of location 1 vs. 2, although it is consistent with aggregate GMD.
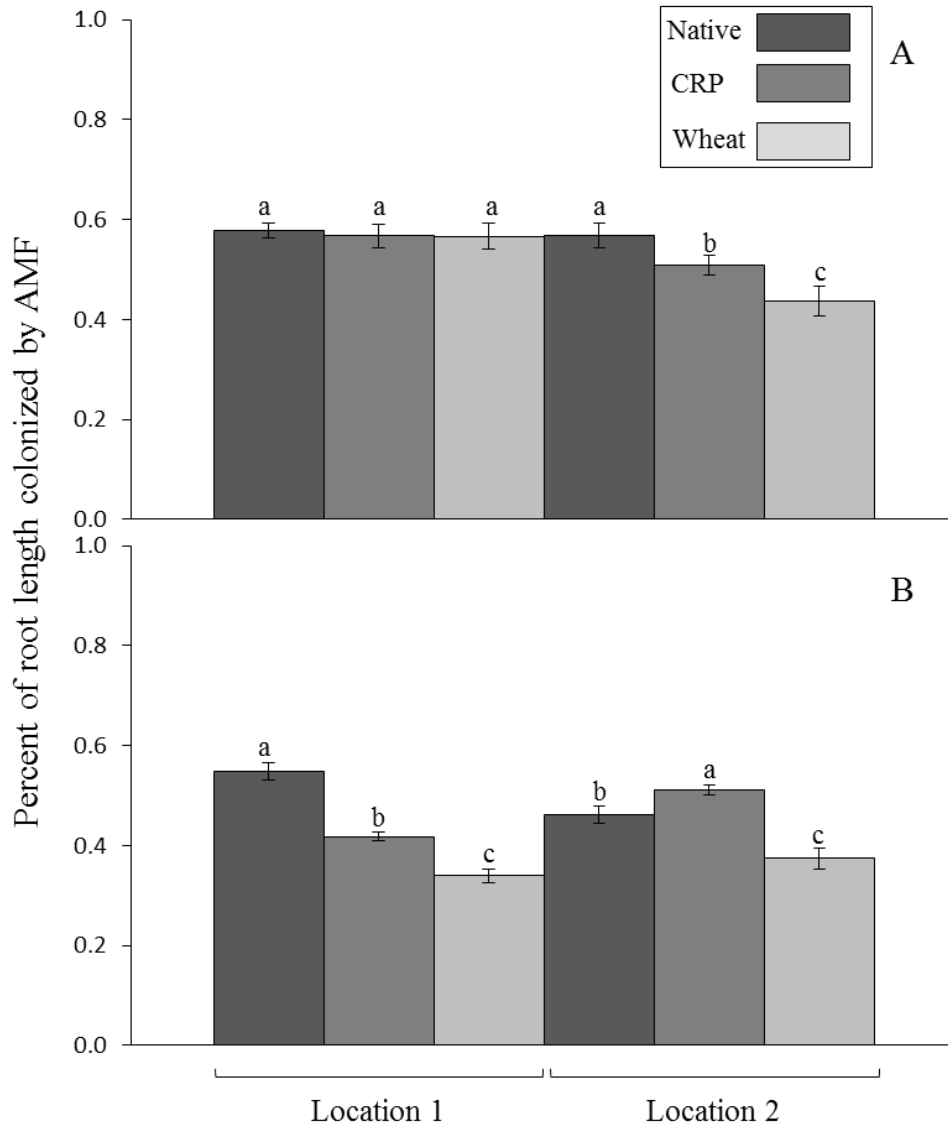
Figure 10 AM fungal intramatrical colonization (percent of root length) +/- SE of Native, CRP and Wheat treatments at each sampling: A) Spring 2010, B) Summer 2010. Different letters indicate means are statistically different according to least significant difference test (p<0.05; n=10).

CHAPTER VI

DISCUSSION

Soil nutrient analysis

It has been well documented that OC levels are relatively stable in undisturbed soil.

However, soil organic matter content, including those of SOC and TN, are quickly

depleted with disturbance (e.g., tillage), and relatively slow to rebuild even if the

disturbance has ceased. Lal (2002) estimates that the gross rate of soil organic carbon

(SOC) sequestration in midwestern rangeland varies from 500 to 800 kg/ha/year in humid

regions following conversion of tilled cropland to conservation practices. Gebhart et al

(1994) found that loamy textured soils under CRP for five years in the Midwestern US

gained an average of 7.9 metric tons C ha^{-1} (7000 lbs C ac^{-1}) to a depth of 40 cm. In this

study, OC and TN content were significantly higher in the Native system than in CRP or

Wheat fields, which is consistent with findings reported in the literature. It remains

unknown how long it may take for SOC and TN to rebuild to pre-cultivation levels

especially as it can vary based on climate and soil type, but it is generally accepted that it

will be a very long time. Following the measurement of SOM and SOC in a

chronosequence of 31 different fields in western Minnesota that had been cultivated for at

least 20 years before switching to native perennial grasses, McLauchlan et al (2006)

estimated that it takes 50-75 years after ceasing cultivation to recover to pre-cultivation

levels based on evaluation of OC, TN, and organic matter content. Data obtained in this study showed that after approximately six years of soil conservation, OC and TN content in CRP had not significantly increased. However, after at least 20 years of ceasing cultivation in the Native fields, OC and TN levels were significantly higher (58.5% higher OC in Native compared to Wheat, and 66.7% higher TN, respectively).

Data on soil pH showed that conventional crop cultivation led to lowering of soil pH, which is counteracted with decrease in fertilizer addition and establishment of perennial vegetation. Extractable P and N content were also much higher in the croplands compared to the soil systems, possible due to chemical fertilizer application in the wheat field. Native and CRP fields also contained some pieces of carbonate, which would suggest that pH values of these soils would likely be higher than a typical Kirkland soil that tends to be very acidic. Extractable P in Native soils at Location 2 were significantly lower than those at location 1, suggesting that occasional grazing resulted in P leaving the system at location 1 via "exported" feces (Harrison, 1985). The Native and CRP fields at location 2 with elevated levels of extractable P also had lower levels of NH_4 compared to the other locations. Concentrations of NO_3-N were the highest in the Wheat fields, followed by CRP, and lowest in Native. The obtained data suggest that conservation practices promoted formation of more stable forms of N, but not the active N pool.

Aggregate Stability

There was a clear trend that showed disturbance (e.g., cultivation/tillage) resulted in lower percentage of water-stable aggregates expressed as GMD. With soil conservation, GMD values in CRP were significantly higher than the wheat field. Although, there was

some variability in GMD depending on sampling time/season or location, the difference in GMD between locations 1 and 2 in the Native system followed a similar trend with the abundance of AMF. Although AMF abundance was consistently high in the CRP fields, often significantly higher than in the Native fields, it did not translate into greater water-stable aggregation. It has long been recognized that fungi plays a crucial role in soil aggregate formation and their abundance is regulated by resource availability. The quantity of water-stable aggregates, however, is governed by their formation as well as accumulation of aggregates. The former is directly linked to AMF abundance; while the latter is affected by time. Data obtained in this study suggest that time is an important factor in enhancing aggregate stability. While the CRP had a relatively high proportion of larger water-stable aggregates, it had a much higher proportion of easily-erodible soil that was more similar to the Wheat treatment than the Native.

Soil Microbial Community Structure Analysis

Levels of 16:1ω5c in all treatments were typically inversely proportionate to the levels of phosphorous in the soil, which is consistent with previous studies (Nagahashi et al, 1996, Douds et al, 2000). At location 1 Native field, phosphorous levels are relatively high compared to that at location 2, and the 16:1ω5c at location 1 is significantly lower than that at location 2. Low soil phosphorous concentrations are known to promote hyphal growth and induce root exudates that promote branching in AM fungi, while higher levels of phosphorous will actually inhibit mycorrhizal colonization (Nagahashi et al, 1996, Douds et al, 2000). This trend was also found in the CRP fields, where location 1 had high P levels and lower AM fungal abundance compared to the CRP at location 2 which

had low P and high AM fungal abundance. All tested Wheat fields had low levels of AMF, which coincide with the high level of both disturbance and phosphorus

Saprophytic fungi were generally higher in the Native fields compared to those in the CRP and Wheat systems, with the exception of the Native field at location 1 which seemed to be subject to seasonal fluctuations, with abundance lower during spring and then higher during summer.

Location 2's Native field showed a consistent trend of higher levels of AMF ($16:1\omega5c$) and saprophytic fungi, total abundance, and water-stable aggregates, but significantly lower levels of disturbance and nutritional stress compared to the Native field at location 1. A potential reason for this could be if the Native field at location 1 has had a more recent history of disturbance compared to location 2; both locations are known to be undisturbed for at least the last 20 years, but perhaps location 1 had been in a more damaged or eroded state when it was removed from crop production. Location 1 (both Native and CRP plots, although there was a greater difference between the Native plots) did have a slightly higher level of nutritional stress, but did not seem to have any evidence of recent disturbance.

Based on total microbial abundance data, it appears that 5-6 years of soil conservation has begun to enhance and stimulate the microbial community, however water-stable aggregation, SOC and TN may be slower to catch up to the same levels as the Native fields.

Using the selected indicators for soil health and restoration, we were able to discern that there was a significant difference between the two Native locations, but less differences

in Wheat or CRP between the two locations. In terms of "recovery" as defined by the parameters evaluated in this study, CRP is on a path to soil ecosystem restoration. Six years of conservation led to about 50% (location 1), or 10% (location 2) recovery compared to the Native fields at each location, respectively (Figure 12).
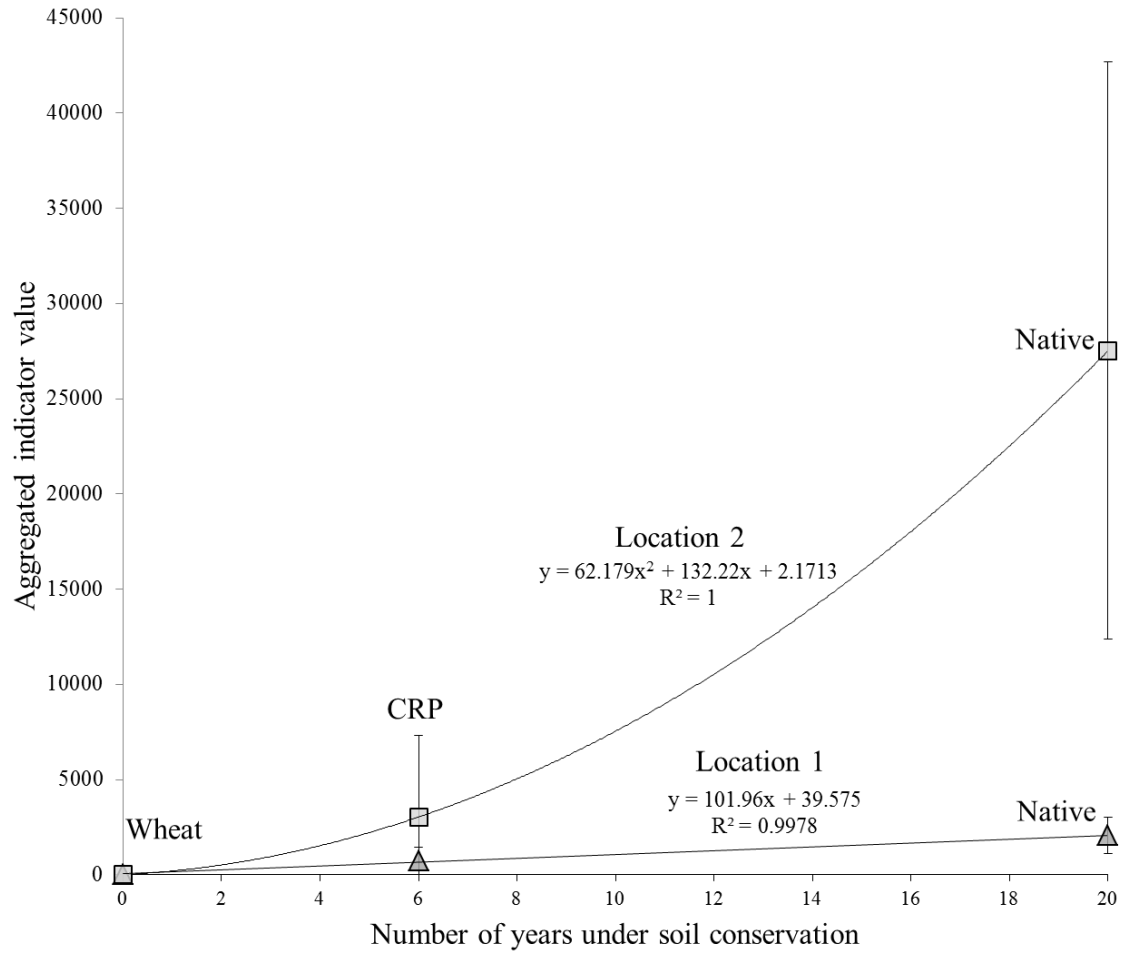
Figure 11 Aggregated means from soil quality indicators (GMD, 16:1ω5c, total abundance, and inverse of ratios for disturbance and nutritional stress) during two sampling periods in 2010 at locations 1 and 2 from Wheat, CRP, and Native plots.

CHAPTER VII

CONCLUSION

The indicators used in this study were sensitive to disturbance levels and nutritional

stress, and revealed differences between systems evaluated. Based on relative comparison

of soil health indicators in Native, CRP, and Wheat systems at two different locations for

two consecutive years, it is clear that the microbial communities of CRP has begun to

shift to more closely resemble Native, with SOC, TN, water-stable aggregation,

disturbance and nutritional stress lagging behind. This suggests that the tested indicators

have different sensitivities to changes in the environment and denotes the requirement of

different amount of time for recovery. In terms of "recovery" as defined by the

parameters evaluated, six years of conservation led to about 50% (location 1), or 10%

(location 2) recovery compared to the Native fields at each location, respectively. It is

clear that six years of conservation was not long enough to restore the soil ecosystems to

a "Native" state. A typical contract with the CRP lasts 10-15 years. More time may be

required for the marginal land to return to its pre-cultivated conditions.

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Institution: Oklahoma State University   Location: Stillwater, Oklahoma

Title of Study: USING MICROBIAL INDICATORS TO ASSESS SOIL ECOSYSTEM
                RESTORATION

Pages in Study: 49   Candidate for the Degree of Master of Science

Major Field: Plant and Soil Sciences

Scope and Method of Study: Microbial and physical soil parameters were determined to
    assess soil ecosystem restoration under a federal conservation reserve program
    (CRP). Three adjacent soil ecosystems were evaluated, including continuous
    winter wheat (*Triticum aestivum* L) production, undisturbed Native (>20 years),
    and marginal land that was formally cultivated and has been under CRP for six
    years. The latter two ecosystems contained similar mixed perennial vegetation.
    Soils were taken from these three ecosystems at two different locations twice a
    year for two consecutive years and were evaluated for water-stable aggregation,
    and composition and structure of the soil microbial community using fatty acid
    methyl ester analysis. Roots isolated from soils were evaluated for arbuscular
    mycorrhizal intraradical colonization.

Findings and Conclusions: The indicators used in this study were sensitive to disturbance
    levels and nutritional stress, and revealed differences between systems evaluated.
    Results show that arbuscular mycorrhizal abundance was high in CRP compared
    to both wheat and native fields. The level of disturbance and nutritional stress
    remained significantly higher in CRP than Native in most of the samples tested,
    indicating less resistance against heat or drought stress. Total microbial
    abundance and geometric mean diameter in CRP systems were significantly lower
    than Native, but significantly higher than the Wheat fields. Data suggested that
    the microbial communities of CRP has begun to shift to more closely resemble
    Native, with content of soil organic carbon and total nitrogen, water-stable
    aggregation, and disturbance and nutritional stress lagging behind. This suggests
    that the tested indicators have different sensitivities to changes in the environment
    and denotes the requirement of different amount of time for recovery. Results
    indicated that six years of conservation led to about 50% (location 1), or 10%
    (location 2) recovery compared to the Native fields at each location, respectively.
    It is clear that six years of conservation was not long enough to restore the soil
    ecosystems to a "Native" state. A typical contract with the CRP lasts 10-15 years.
    More time may be required for the marginal land to return to its pre-cultivated
    conditions.

ADVISER'S APPROVAL: Dr. Shiping Deng