SOIL AGGREGATE CARBON STORAGE IN RESTORED TALLGRASS PRAIRIES: A COMPARATIVE ANALYSIS BETWEEN A FORMERLY CULTIVATED SITE LOCATED ON NATIVE PRAIRIE SOIL AND AN ARTIFICIALLY CREATED LANDSCAPE

A THESIS SUBMITTED TO THE FACULTY OF THE PROGRAM IN PLANT BIOLOGY AND CONSERVATION

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ABSTRACT

Tallgrass prairie restorations may act as sustainable carbon sinks via the integration of soil organic matter into water-stable soil aggregates. Although studies at formerly cultivated sites located on native prairie soil have documented increasing soil aggregation and carbon storage with age, there are few comparable studies in non-native systems. As a result, this study investigated the following: is an artificially created prairie able to achieve similar below-ground functioning relative to a formerly cultivated native counterpart? A comparative analysis of two Chicagoland tallgrass prairies was undertaken, which consisted of a 26-year-old artificially created site constructed on former marshland verses a 34-year-old restoration located on native prairie soil that was cultivated for approximately 150 years. In particular, the study measured the correlation between water-stable macroaggregate soil carbon storage (>425 μm diameter) with multiple soil indicators such as arbuscular mycorrhizal fungi (AMF) root colonization, root biomass, root substrate quality, and soil bulk density. The results indicated an increase in macroaggregate carbon storage with age at the formerly cultivated site (Fermilab), ($p<0.001$) in contrast to no apparent increase at the artificially created site (CBG). Similarly, AMF root colonization exhibited a statistically significant increase at Fermilab ($p<0.01$) but not at CBG. Interestingly, above-ground succession at CBG, which has exhibited a decline in warm season grasses that depend on AMF associations, corroborated the absence of an increase. No significant differences were observed between either bulk root biomass or fine root substrate quality (<0.2 mm diameter). Lastly, soil bulk density exhibited an increase at Fermilab ($p<0.001$) verses a decrease at CBG ($p<0.001$), which is counterintuitive to the trend in macroaggregate abundance at each site. Overall, CBG might differ from Fermilab due to its non-native soil origin, disturbances incurred during construction, and/or hydrological issues. The results highlighted the importance of site history, as non-native prairie soil and/or excavation activities might impose constraints on traditional ecological succession.

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INTRODUCTION

The Prairie Ecosystem and the Soil Carbon Cycle

The North American prairie represents a critically endangered ecosystem, as 99.9% of its land area was converted during the 19th and 20th centuries (Ladd, 1995). In the Midwest region, tallgrass prairie restoration affords the opportunity not only to reintroduce native vegetation and organisms, but also to raise environmental awareness and to counteract greenhouse gas (GHG) emissions by storing organic carbon within the soil (Conant et al., 2001; Post & Kwon, 2003; Lal et al., 2003; Matamala et al., 2008). Prairie restoration projects are able to act as net carbon sinks when net primary production (NPP) exceeds the losses from decomposition, erosion, and the carbon cost associated with land management.

Organic carbon enters the terrestrial landscape via photosynthesis as light energy catalyzes the conversion of atmospheric CO_2 into simple sugars. These sugars are the fundamental energy source that drives the soil carbon cycle. In tallgrass prairie, the majority of carbon is stored within partially decomposed plant, animal, and microbial residues termed soil organic matter (SOM) (Paul, 2007). Once plant matter dies and returns to the soil, carbon storage and the residence times of organic materials are modulated by various factors such as soil texture (Brye & Kucharik, 2003), chemical stability (Paul, 2007), resistance to microbial attack (Paul, 2007), and soil nutrient levels (Asner et al., 1997). SOM that is integrated into recalcitrant fractions, particularly water-stable aggregates, are less prone to decomposition (i.e., microbial oxidation) and/or erosion than labile organics in bulk soil (Jastrow, 1996; Six et al., 2000; Goebel et al., 2009). One study in a Midwestern tallgrass prairie recorded macroaggregate (>212 um diameter) and microaggregate (53-212 um diameter) residence times of 140 years and 412

years, respectively. (Jastrow et al., 1996). Although macroaggregates exhibit a shorter residence time in the soil, they report greater carbon content per unit weight as a result of differing organic compound composition (Jastrow et al., 1996; Puget et al., 2008).

Soil aggregates are "clumps" of soil that are a composite of partially decomposed organic matter such as lignin, chitin, and pectin that are chemically bonded to fine, inorganic clay or amorphous particles (Tisdall & Oades, 1982). They are held together by sticky exudates produced by roots and microbes, preserved within a matrix of roots and fungal hyphae (Coleman & Crossley, Jr., 1996). Soil aggregates are valuable because they act as a carbon reservoir, promote aeration, create microhabitats for soil organisms, and prevent leaching via binding micronutrient cations such as Ca^{+}, Zn^{+}, and K^{+} (Nardi, 2007). The accumulation and preservation of aggregates over a restoration period are linked to nutrient cycles and heterotrophic foodwebs, which, in turn, are linked to above-ground ecological processes (Paul, 2007).

Soil Carbon Cycling in Restored Tallgrass Prairies

Tallgrass prairie is the native, dominant ecosystem of the Midwest, and restorations located on native prairie soil may exhibit a trajectory towards remnant prairie conditions (Potter et al., 1999; Knops & Tilman, 2000, Conant et al., 2001). Previously cultivated sites are relatively degraded in SOM since conventional agricultural methods such as tillage break up the soil aggregates and facilitate the microbial oxidation of organic matter (Waters & Oades, 1991), while inadequate root and fungal structure render the organic matter susceptible to erosion (Nardi, 2007). SOM fractions decompose on unique timescales, with preferable oxidation of the binding agents that glue macroaggregates together (Waters & Oades, 1991), as they are more palatable and accessible. Factors such as soil texture and restoration management influence the

rate of degradation and upwards to 50% of SOM may oxidize or erode in the top 20 cm after 30-50 years of continuous cultivation (Lal, 2003). Restoration activities and time enable the accrual of above- and below-ground carbon stocks.

In regard to tallgrass prairie vegetation on non-native soils, restoration activities are most likely met with varying degrees of success, as SOM degraded systems on native prairie soils are variable within themselves. Although the definition of a successful prairie restoration is debated, they are commonly indexed by characteristics such as an increase in plant species diversity, dominance of warm-season grasses, reestablishment of soil invertebrates and native pollinators, as well as an increase in soil aggregation with restoration age (Betz, 1996). Depending on the nature and magnitude of the disturbance(s), therefore, below-ground ecosystem processes might be modulated in non-native system (Milesi et al., 2005; Scharenbroch et al., 2005; Kaye et al., 2006; Golubiewski, 2006; Lorenz & Lal, 2009).

To date, very few studies examined the difference between below-ground ecosystem processes on sites with and without a history of a prairie vegetation. In addition, in the context of carbon storage, the recovery rate of carbon stocks other than bulk soil has not been adequately addressed. Previous research estimated that it might take over 50 years for soil aggregates to reach remnant prairie levels (Jastrow, 1987) and up to 100 years for fungal carbon stocks to equilibrate (Matmala et al., 2008). Although provocative, the conclusions of these preliminary studies cannot be extrapolated.

Factors that Influence Water-Stable Aggregate Formation and Carbon Storage

Water-stable aggregate formation is driven by physical, chemical, and biological factors that interact above- and below-ground and across spatial and temporal scales. Holding climate

and inorganic nutrient availability constant, the predominant regulatory controls are fungi, roots, and physical soil parameters (Rillig & Mummey, 2006).

a) Fungi

Fungi account for approximately 50% of the organismal biomass in prairie soils and, therefore, fundamental to soil carbon cycling and aggregate formation (Rillig & Mummey, 2006). The two main types in tallgrass prairie are saprotrophic and arbuscular mycorrhizal fungi, which decompose organic matter and form mutualisms with plant roots, respectively.

Saprotrophic fungi are the principle decomposers of SOM (Rillig & Mummey, 2006). Since prokaryotes and other microorganisms have greater access to soluble compounds near the soil surface, saprotrophs are equipped with enzymes to degrade recalcitrant compounds such as lignin, chitin, and glomalin (Rillig & Mummey, 2006). Enzymes break apart macromolecules to obtain residues that can be absorbed and/or metabolized (Sinsabaugh et al., 2002) and chemically complex organic byproducts bind to inorganic minerals such as clay to form microaggregates (often defined as ~2-250 μm diameter) (Paul, 2007). At the same time, microbial exudates facilitate the construction of macroaggregates (often defined as >250 μm diameter) via gluing microaggregates together. The cumulative effect may create long-lived organic compounds in the soil.

Arbuscular mycorrhizal fungi (AMF) form mutualistic relationships with plant roots in 84% of grass species (Newman & Reddel, 1987) and, on a broader scale, approximately 70% of terrestrial plants (Paul, 2007). AMF penetrate the cell walls of the root cortex and supply nutrients such as phosphorous, nitrogen, and water in exchange for photosynthetic carbohydrates (Rillig, 2004). Arbuscule and coil structures act as the site of exchange, while fungal filaments

called hyphae grow into tight pore spaces to capture micronutrients and shuttle them to their hosts. Plants yield 5-30% of their photosynthate to AMF, which is stored in vesicles that branch off hyphae (Coleman & Crossley, Jr., 1996). Previous research concluded AMF species diversity to correlate with above-ground plant biodiversity, with important implications for succession (van der Heijden et al., 1998); therefore, AMF recovery rate was critical for the healthy above- and below-ground functioning of prairie restorations.

AMF hyphae act as an intermediary to soil aggregate accumulation, as they produce glomalin. a glycoprotein exudate that glues together microaggregates (Wright & Upadhyaya, 1998; Rillig et al., 2002). In tallgrass prairies, hyphal length has been shown to correlate with water-stable aggregate diameter (Jastrow, 1990). In addition, a correlation was found to exist between plant species composition and AMF abundance (Rillig et al., 2002). Grasses tend to have a greater affinity to develop AMF mutualisms relative to forbs; however, inorganic nutrient availability (Egerton-Warburton & Allen, 2000; Egerton-Warburton et al., 2007) and region (Schultz et al., 2001) modulate dependency. Whereas warm season grasses such as *Andropogon gerardii* (big bluestem, Poaceae) and *Sorghastrum nutans* (Indian grass. Poaceae) require AMF to thrive, cool season species such as *Elymus canadensis* (Canada wild rye, Poaceae) do not (Packard & Mutel, 1997). Fungicide treatment studies corroborate these differences, so it seems as though mycorrhizal availability and growth responsiveness are related (Wilson & Harnett, 1997). Overall, prairie grass dominated systems that depend on AMF associations are better at forming aggregates over the restoration period (Jastrow, 1987).

b) Roots

Tallgrass prairie plant roots contribute to aggregate formation and stabilization (Miller & Jastrow, 1990), with root substrate quality being the principle regulatory component of decomposition rate (Silver & Maya, 2001, Matamala et al., 2008). In tallgrass prairie, roots are produced in a 3:1 ratio to shoots (Lad, 1995), but the ratio might increase if prescribed burns are frequently used (Nardi, 2007). After several decades of restoration management, root biomass may return to remnant prairie levels (Tilman et al., 2001; Matamala et al., 2008). Root structures intermesh with aggregates to prevent erosion (Barthes & Roose, 2002), as root tips exude binding agents such as polysaccharides that facilitate the accumulation of macroaggregates (Angers & Giroux, 1996; Puget et al., 2000). In addition, root length and AMF root colonization have been shown to correlate with the diameter of water-stable soil aggregates (Miller & Jastrow, 1990).

Root matter substrate quality has the ability to modulate soil decomposition rate in grasslands (Silver & Maya, 2001). Multiple factors determine substrate quality such as diameter., age, strength of chemical linkages, toxicity of soluble byproducts, and micronutrient levels.Carbon:nitrogen ratio (C:N) is commonly used to predict decomposition rate; roots less than 2 mm in diameter are nitrogen-rich (lower C:N) and tend to decompose quicker, with a turnover rate measured on a timescale of days to months (Silver & Maya, 2001). In one study the average C:N ratio for graminoids was estimated as follows:, 67 for <2 mm, 104 for 2-5 mm, and 156 for >5 mm. The concentration of lignin and other recalcitrant compounds serve as a principle component.

In tallgrass prairie, nitrogen is often rate-limiting, as microbes and plants compete for plant-available forms such as ammonium and nitrate (Paul, 2007). The terrestrial carbon and nitrogen cycles are linked and modulate heterotrophic foodwebs and aboveground productivity

(Asner et al., 1997). In general, when nitrogen-rich SOM is abundant in the soil, the chemical conversion into plant-available forms may favor soil carbon storage (Chapin III, 2002). Also, substrate quality is believed to be a necessary condition for soil carbon storage in the absence of inorganic nutrient deposition (Waldrop et al., 2004).

c) Physical Soil Parameters

Physical soil parameters set constraints on soil carbon storage and aggregation in prairies; for example, fine textured prairies soils have been shown to store a greater concentration of SOM than coarser soils (Brye & Kucharik, 2003). Clay enhances the accumulation of long-lived organics (e.g., humic and fulvic acids within aggregates) by binding organic matter and, to a lesser degree, microbial enzymes (Chapin III, 2002). The binding of organic matter occurs because clay particles have a high concentration of negatively charged sites, which attracts positively charged amine groups on recalcitrant organic byproducts. In addition, polyvalent cations may bind to the surface of clay and form bridges that attract negatively charged carboxyl groups. In both instances, the end result is the formation of microaggregates.

Soil density influences a number of processes including soil hydrology, aeration, root penetration, and leaching of micronutrients (Nardi, 2007). In general, clayey soils tend exhibit lower bulk densities due to larger internal surface areas (Paul, 2007). However, clayey soil may have a higher bulk density when severely compacted, with consequences on below-ground processes; pore spaces less than ~5 μm restrict the entrance of hyphae and enzymes as well as limit aerobic respiration (Ritz & Young, 2004). Other soil properties influence soil nutrient cycles and below-ground functioning. For instance, soil salinity may modulate AMF associations because high salinity levels adversely affect hyphal growth and spore germination (Juniper &

Abbot, 1993; McMillen et al., 1998). Also, in some instances, neutral soils exhibit faster decomposition rates relative to acidic soils (Chapin III, 2002). Acidification causes cation leaching and the build up of organic acids in SOM that may hinder the accumulation of organic matter in the soil (Chapin III, 2002). Overall, it has been proposed that the interaction of ecosystem processes in the context of above- and below-ground functioning is dictated by a number of state factors: climate, parent material, potential biota, topography, and time (Jenny, 1982).

Research Significance

Tallgrass prairie restorations are increasingly popular because they provide an array of benefits to the environment as well as humans. In the context of global warming, carbon accrual might offset GHG emissions, albeit to a small degree. To assess the efficacy of carbon storage, the water-stable aggregate fraction might serve as a proxy as it exhibits a slow turnover rate, measured on the order of hundreds of years (Jastrow et al., 1996). More importantly, soil aggregates are associated with healthy ecosystem functioning: porosity is increased, leaching of cations is prevented, and microhabitats are created (Nardi, 2007). Overall, the goal of this research was to predict soil macroaggregate carbon storage (>425 μm) over several decade of restoration management at an artificially created site located on marshland soil with a formerly cultivated site located on native prairie soil. The topic is timely as municipalities explore options to mitigate carbon emissions and integrate native habitat within their communities.

OBJECTIVES

This study compared water-stable macroaggregate carbon storage (>425 μm) at an artificially created site on marshland soil to a formerly cultivated site located on native prairie soil. Specifically, factors such as AMF root colonization, root biomass, root substrate quality, and bulk density were investigated in the context of macroaggregate carbon storage. At least two reasons implicate the macroaggregate fraction: first, macroaggregates are greater in carbon concentration because they are held together by carbon-rich root and microbial exudates that enable the accumulation of larger soil aggregates (Jastrow et al., 1996), and, second, macroaggregates exhibit a turnover rate on the scale of hundreds of years when root and hyphal structure are not lacking (Rillig & Mummey, 2006).

For the comparative analysis, I employed dissimilar sampling methodologies due to restrictions in data availability. For CBG, I analyzed archived, frozen soil cores, whereas at Fermilab I used the chronosequence technique. Each method may provide an accurate depiction of activity over time. However the robust nature of this comparison is uncertain. Knops and Tilman (2000) suggest that they are interchangeable, but too few studies have employed a comparative analysis between the two techniques, so it is not possible to corroborate its validity.

Specifically, three questions were addressed:

(1) Relative to a formerly cultivated site located on native prairie soil, how does an artificially created site constructed on marshland soil differ in its ability to store soil macroaggregate carbon over several decades of restoration management?

(2) How does AMF root colonization, root biomass, root substrate quality, and bulk density correlate with soil aggregate carbon storage?

(3) What do the results suggest for municipalities that are interested in offsetting GHG emissions associated with global warming?

Hypotheses:

(1) Soil macroaggregate carbon storage (>425 μm) will exhibit a trajectory towards tallgrass remnant prairie conditions. A body of literature has established that prairie restoration projects constructed on SOM degraded sites will exhibit an increase in soil aggregation and SOM concentration with time (Lal, 2003; Post & Kwon, 2000).

(2) Arbuscular mycorrhizal fungal root colonization will increase with soil macroaggregate carbon storage over the restoration period. Fungal hyphae contribute directly to aggregate formation and represent a principle carbon input via the secretion of binding agents such as glomalin (Wright & Upadhyaya, 1998). Support for this expectation comes from chronosequence studies at Fermilab that have shown increases in both water-stable aggregate abundance (Jastrow, 1987) and microbial biomass (Matamala et. al, 2008) with restoration age.

(3) Root biomass will increase with soil macroaggregate carbon storage over the restoration period. Previous research at Fermilab estimated that root biomass recovers within several decades of restoration management (Matamala et al., 2008), and a direct correlation exists between root length and aggregate diameter (Jastrow et al., 1996). In addition, it was expected that root substrate would become more palatable with age (lower C:N) as fine root hairs (<2 mm diameter) become more concentrated in the soil.

(4) Soil bulk density will alleviate with restoration age due to the synergistic effect of soil macroaggregate abundance, root biomass, and mycorrhizal colonization. The establishment of prairie vegetation is associated with a decrease in bulk density because roots and AMF associations facilitate aggregate development (Nardi, 2007).

MATERIALS AND METHODS

Study Sites

This study was a comparative analysis between the Chicago Botanic Garden Suzanne S. Dixon Prairie (CBG) and the Richard F. Betz Fermilab Prairie (Fermilab) (Figure 1). CBG was established on former marshland, whereas Fermilab was planted on formerly cultivated, native tallgrass prairie.

The first site, CBG, is a 6-hectare (ha) prairie restoration in Glencoe, IL (42^{o} 09' 07" N, 87^{o} 47' 11" W, Figure 2). The site was planted in 1982, and was classified as "artificially created" because it was constructed on highly disturbed marshland soil. During construction in the 1960s, bulldozers and other heavy machinery were used to excavate and sculpt the terrain, and approximately 6 inches of imported topsoil were applied over a mostly clay subsurface (Figure 3). The topsoil (0-10 cm) was classified as mesic clay-loam, and the average inorganic particle composition was 43.9% sand, 7.18% silt, and 48.92% clay (Figure 4). Approximately 250 species are represented, and the predominant plant species include *Solidago altissima* (goldenrod, Asteraceae), *Pycanthemum virginianum* (common mountain mint, Lamiaceae), and *Spartina pectinata* (prairie cord grass, Poaceae). However, plant species succession has not behaved in a predictable fashion. Despite a prescribed burn regime and other management

activities, efforts to promote a shift towards warm season grasses and conservative prairie species have been unsuccessful. In general, there has been a decline in C4 graminoid abundance and diversity over the past 10-15 years (D. Sollenberger, CBG, pers. comm.).

Average daily high temperature during the growing season (April – October) from 1991-2008 was 22.32 ^{o}C (Figure 5), and ranged from 19.89 ^{o}C in 1992 to 23.78 ^{o}C in 2002 (Figures 6-7). For 2008, daily average high temperature was 22.38 ^{o}C, 0.06 higher than the average for all years. The average number of days above 32.2 ^{o}C (90 ^{o}F) was 15.2, and ranged from three days in 1992 to 30 days in 2002. In 2008, six days exceeded 32.2 ^{o}C, which was nine days less than average.

The driest growing season occurred in 2005, while the wettest was 2001 (Figures 8-9). The average total precipitation per year was 64.82 cm, and ranged from 32.39 cm in 2005 to 92.48 cm in 2001. The average monthly precipitation was 9.3 cm, with the highest total average at 13.2 cm in 2001 and the lowest at 4.6 cm in 2005. For 2008, total precipitation was 80.5 cm (15.7 cm above normal) and average monthly precipitation was 11.5 cm (2.2 cm above normal).

A number of severe flooding events have occurred since 1982 (date for which data was available). The dates of the most severe events (>10 cm) are as follows: 9/23/1986 - 9/30/1986 (11.3 cm), 8/14/1997 - 8/15/1997 (19.2 cm), 8/10/2000 - 8/21/2000 (19.0 cm), 10/12/2001 - 10/14/2001 (11.2 cm), 8/18/2007 - 8/20/2007 (11.3 cm), and 9/13/2008 - 9/15/2008 (19.4 cm).

The second site, Fermilab, is comprised of a series of restored prairies located at the Fermilab National Environmental Research Park in Batavia, IL (41^{o} 50’ 30” N, 88^{o} 14’ 30” W, Figures 10-11). The site is located on former tallgrass prairie that was continuously cultivated from 1850 to 1960. The restoration project commenced in 1975, and as of 2002, 452 ha have been restored. The topsoil (0-10 cm) was classified as mesic clay-loam, and the average

inorganic particle composition was 40.10% sand, 11.38% silt, and 48.53% clay (Figure 12). Each plot has exhibited similar above-ground ecological succession, with a steady shift towards C4 graminoids. As of 1996, approximately 90 prairie species were identified in the 34-year-old plot (Betz, 1996). Predominant species include *Sorghastrum nutans* (Indian grass, Poaceae) and *Andropogon gerardii* (big bluestem, Poaceae), amid a diverse mix of C3 species and prairie conservative plants. A remnant prairie exists on the northeast boundary of the property along a railroad right-of-way. The site was not actively managed by Fermilab, but it could be used a proxy for native conditions even though herbicide has been applied over the last decade.

Since 1995, the average temperature during the growing season was 16.7 ^{o}C, (Figure 13) and ranged from 15.6 ^{o}C in 1996 to 18.0 ^{o}C in 2005 (Figures 14-15). The average total precipitation per year was 61.3 cm, and ranged from 29.4 cm in 2005 to 86.4 cm in 2002 (Figure 16; Figure 17). The average monthly precipitation during the growing season was 8.8 cm, with the highest value at 12.3 cm in 2002, and the lowest value at 4.2 cm in 2005. Across sites, the largest difference in precipitation was 2001, when CBG received 112.5 cm of precipitation, while Fermilab received 69.3 cm. Relative to CBG, yearly precipitation at Fermilab was less by 3.6 cm, but monthly total precipitation was similar (Fermilab was less by 0.05 cm) (Figure 18).

Fermilab Previous Research Findings

Previous research at Fermilab indicates an above- and below-ground trajectory towards native prairie conditions. The project's founder, Richard F. Betz, and various ecologists at the Argonne Laboratory established a body of literature to monitor ecological succession.

Jastrow (1987) investigated soil aggregate recovery utilizing plots that were 2, 5, 8 and 11 years of age, a cornfield, and a 14-year-old restoration pasture. It was concluded that

approximately 50 years would elapse before aggregates (> 250 μm) attain remnant levels. Also, there was a correlation between warm season grasses and water-stable soil aggregates (200 μm-2000 μm), but the data were unable to tease out the age effect. Miller and Jastrow (1990) found a positive correlation between aggregate size and both root and hyphal length, which reinforced the theory that root and fungal hyphae facilitate the binding of microaggregates to macroaggregates proposed by Tisdall and Oades (1982). Moreover, Jastrow and Miller (1993) sought to determine how plant neighbor species might effect AMF root colonization. The data revealed an interaction between *Andropogon* and forb species, with higher root colonization on *Andropogon* when it was adjacent to *Coreopsis triperis* (Asteraceae) and *Solidago altissima* (Asteraceae), relative to warm-season grass species. However, the authors concede that the differences might be an artifact of age and/or neighbor root biomass. Miller et al. (1995) conducted a comparative analysis of AMF hyphal growth between a pasture and restored prairie during the growing season, and average hyphal length was longer in the restored prairie. During a drought event in spring, the data found that root growth was suppressed, while hyphal length was uninhibited. The authors concluded that hyphae and root morphology are modulated by moisture conditions.

Jastrow et al. (1996) estimated the turnover rate of soil aggregates. Average turnover rate for macroaggregates (>212 μm) was 140 years, while for microaggregates (53-212 μm) it was 412 years. This indicates that microaggregates are more recalcitrant and/or more physically protected from decomposition. In addition, organic carbon concentration was greater in macroaggregates, possibly because the binding agents that glue together microaggregates are more palatable. Schultz et al. (2001) investigated if region may modulate AMF mutualisms with warm season grasses. They concluded that AMF associations with *Andropogon* were less

dependent at Fermilab than a counterpart in Kansas. Specifically, the ecotypes from Kansas were three times more responsive to AMF inoculation, which might have been caused by adaptations to soil nutrient levels.

Allison et al. (2005) analyzed phospholipid signatures for a number of microbes including gram-positive bacteria, gram-negative bacteria, actinomycetes, AMF, and saprotrophic fungi. The lowest AMF values were reported in the cultivated field and increased with prairie restoration age. Although the fungi:bacteria ratio was initially high, it decreased with restoration age. The below-ground succession implications are unclear, but it might indicate dissimilar succession across plots or covariance with another variable. Lastly, Matamala et al. (2008) estimated the recovery of various above- and below-ground carbon stocks. Carbon and nitrogen stocks in aboveground vegetation reached native prairie levels within 17 years, while belowground root carbon and nitrogen stocks recovered after several decades. Similar to the results of Allison et al. (2005), microbial biomass was lowest in the cultivated field and increased with restoration age. They estimated that both total soil carbon and microbial biomass would take upwards to 150 years to recover. Lastly, they found root (<5 mm in diameter) C:N to be lower in the native prairie (~70) when compared to the restored prairies (~90). The results were predicted to be driven by above-ground successional characteristics, which may account for differences in short-term SOM decomposition rate.

Sample Collection

Dissimilar sampling techniques were employed as a consequence of data availability. For CBG, archived soil cores were analyzed, whereas at Fermilab the chronosequence technique was used.

At CBG, soil cores were collected by Dave Sollenberger on dates ranging from mid-fall to early winter for the following years: 1991 (9 growing seasons post creation), 1997 (15 growing seasons post creation), and 2000 (18 growing seasons post creation). In addition, fresh cores were collected on January 8, 2009 (26 growing seasons) using the same methodology. Specifically, soil cores were extracted using a soil auger (7.5 cm diameter; 10 cm deep) from 13 plots spread randomly across transect lines (~150 m long) marked with metal rods (A1-A3. B1-B5, C1-C5). Archived soil was preserved in a conventional freezer at -7 ^{o}C, while 2008 soil was stored in a refrigerator at 4^{o}C.

At Fermilab, soil cores were collected on August 25, 2008. Sampling points were determined using aerial photographs provided by Google Earth and a random number generator set to the dimensions of each plot. In total, 5 soil cores (7.5 cm diameter; 10 cm deep) were sampled randomly across the rhizosphere for each plot. A 1.0 m^2 quadrat was established, and soil cores were extracted from the center point and the rest from the edges of the quadrat. In total, 9 plots were sampled (Figures 4-5). Prairie restorations included those planted in 1975 (34 growing seasons; 3.6 ha), 1977 (32 growing seasons; 11.7 ha), 1981 (28 growing seasons; 6.9 ha), 1984 (25 growing seasons; 13.4 ha), 1988 (21 growing seasons; 27.9 ha), 1990 (19 growing seasons; 34.0 ha), and 2000 (9 growing seasons; 36.0 ha). In addition, samples were collected from an on-site cornfield (zero growing seasons; total ha unknown) and a remnant prairie located along a railroad right-of-way (>150 growing seasons; 0.5 ha). The soil was stored in a laboratory refrigerator at 4^{o} C until analysis.

In addition, root matter (n=3/type/site) was collected in April 2009 for the dominant C3 and C4 species at each site. This included *Solidago altissima* (golden rod, Asteraceae) and

Spartina pectinata (prairie cord grass, Poaceae) at CBG, and *Solidago altissima* (golden rod, Asteraceae) and *Sorghastrum nutans* (Indian grass, Poaceae) at Fermilab.

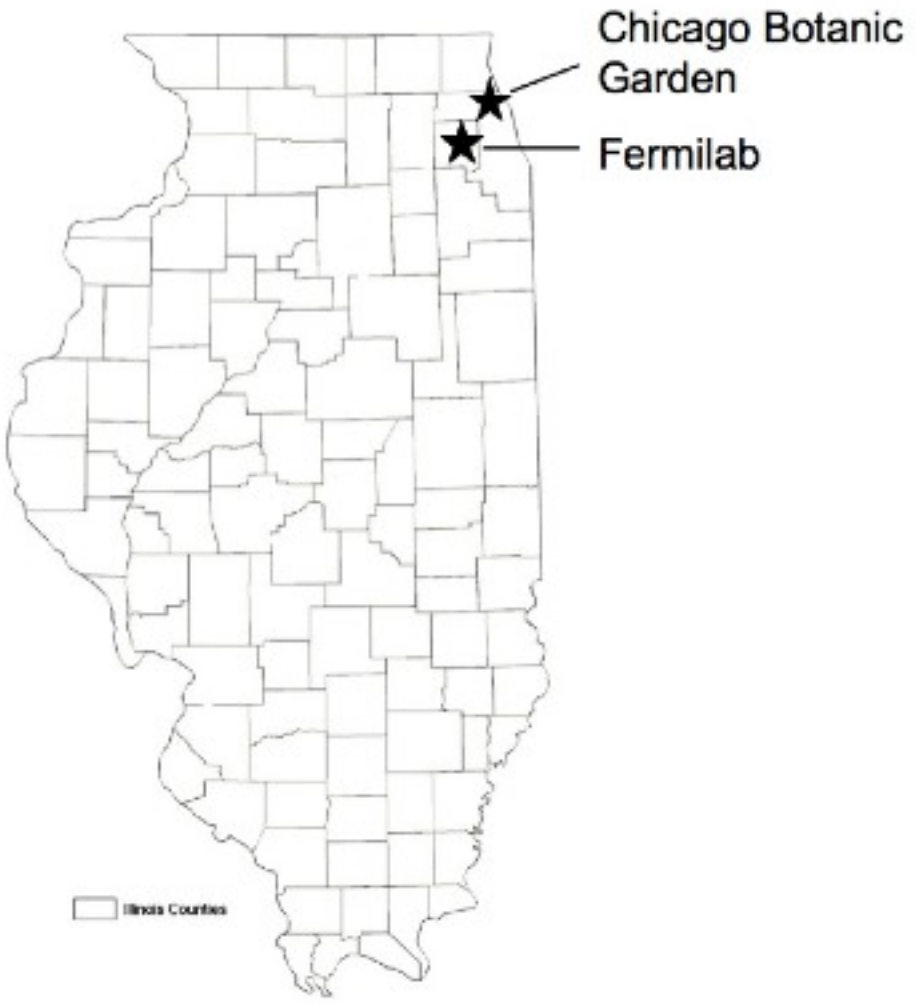

Figure 1. Location of study sites in Cook (Chicago Botanic Garden) and Kane County (Fermilab), Illinois. Map modified from http://www.geographic.org.

Figure 2. Chicago Botanic Garden (CBG) Suzanne S. Dixon Prairie. 42^{o} 09’ 07” N, 87^{o} 47’ 11” W. Arrowed box indicates the area of interest. Modified image from Google Earth.


Figure 3. Construction of Chicago Botanic Garden premises (circa 1960s). The site may be defined as an artificially created prairie due to fact that it was former marshand and due to the application of soil from outside sources. Photo courtesy of David Sollenberger, Chicago Botanic Garden.

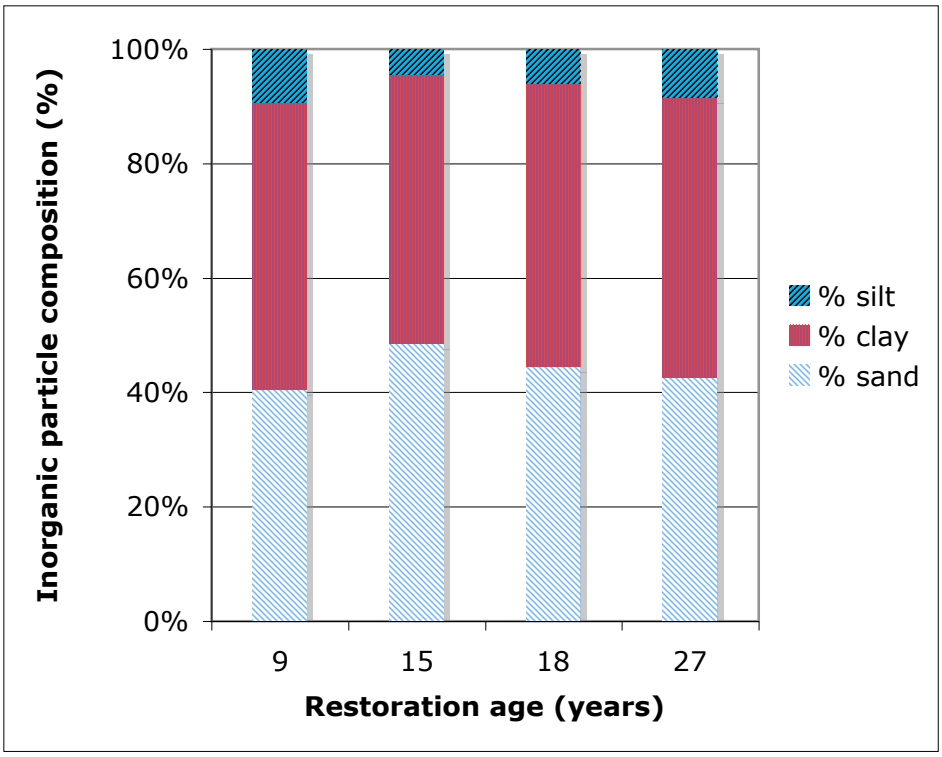

Figure 4. Composition of inorganic soil particles at CBG.

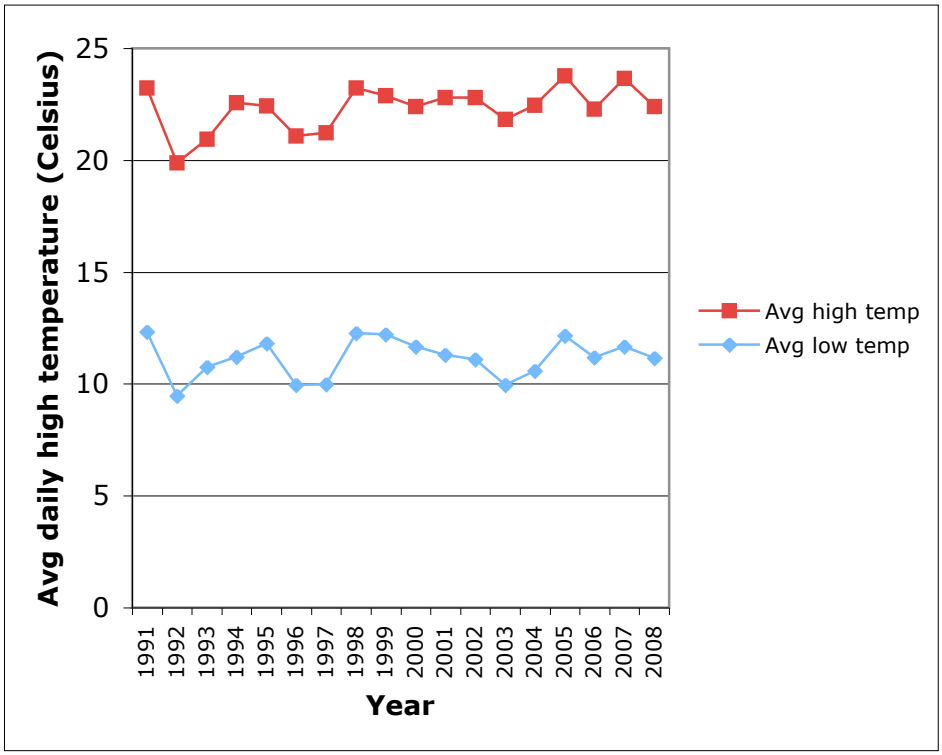

Figure 5. CBG climate data for average daily high temperature and average daily low temperature during the growing season (April-October).

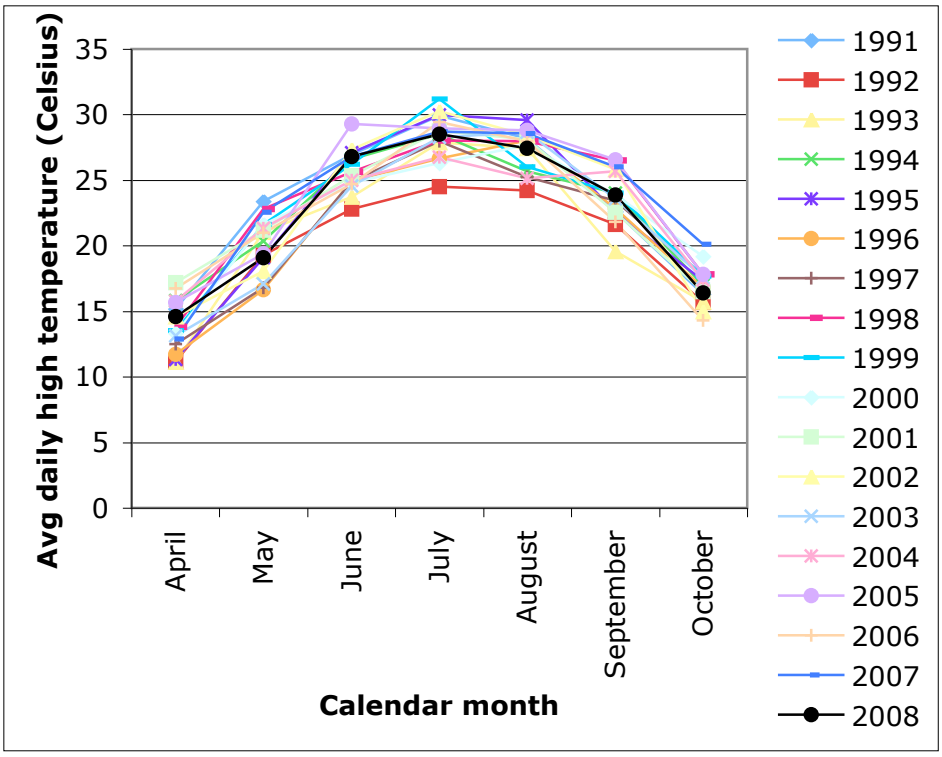

Figure 6. CBG monthly average high temperatures from 1991-2008.

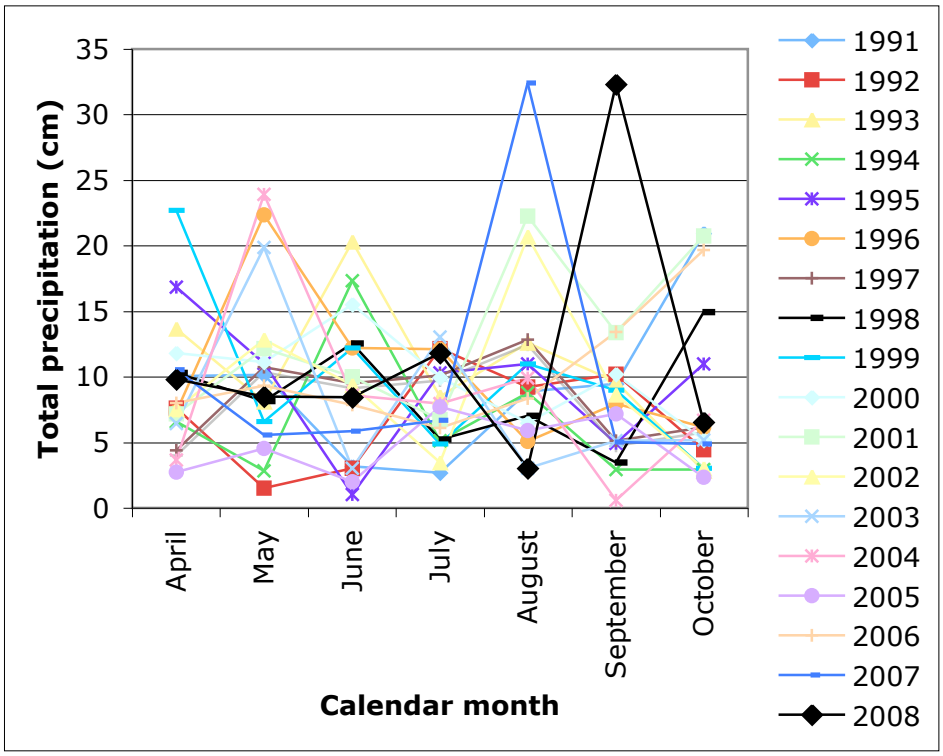

Figure 7. CBG monthly total precipitation from 1991-2008.

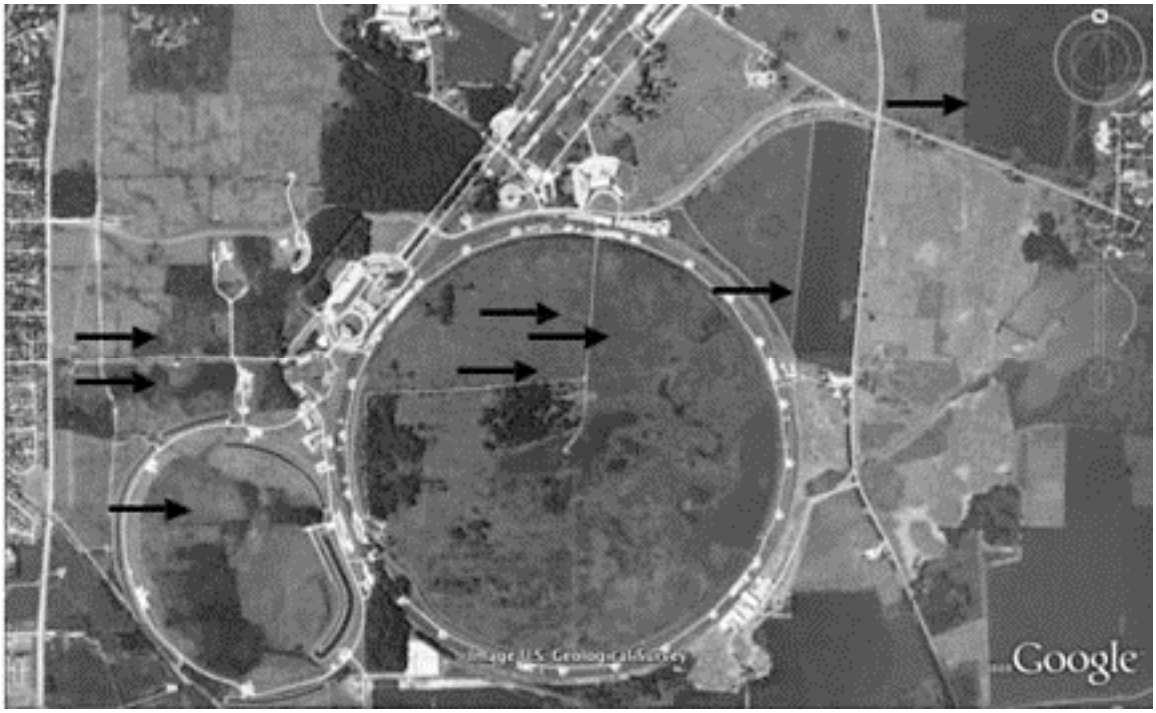

Figure 8. Fermilab National Environmental Research Park Prairie (Fermilab). 41° 50’ 30” N, 88° 14’ 30” W. Arrows indicate approximate location of soil sampling. Note: Off-site remnant site is not included. Modified image from Google Earth.

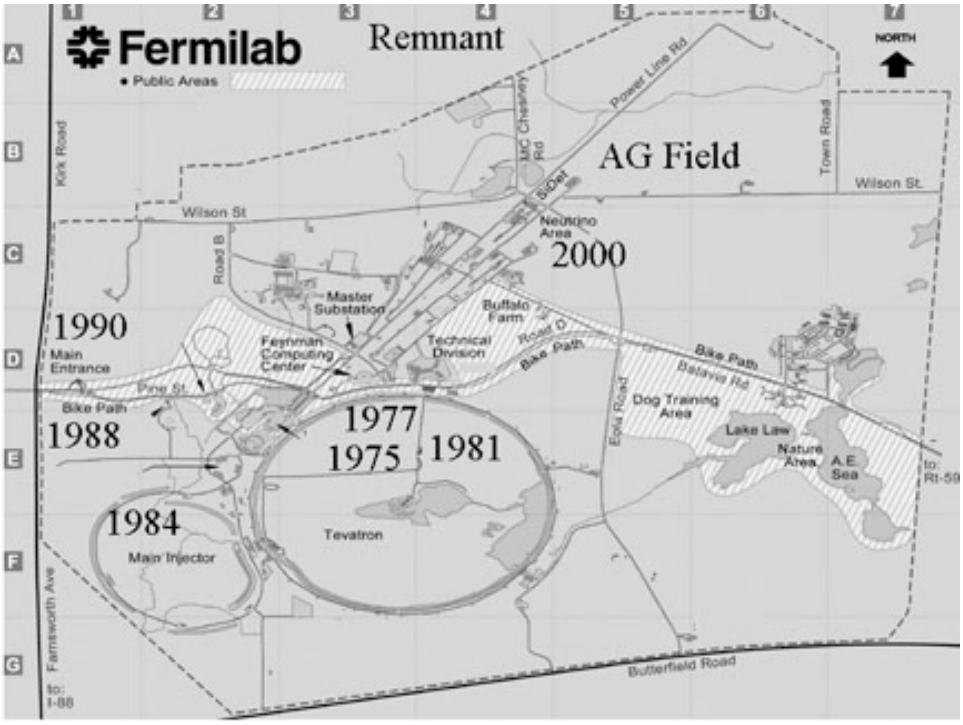

Figure 9. Fermilab chronosequence. The approximate location of each prairie restoration plot is indicated by a corresponding planting date. Modified image from the Fermilab National Environmental Research Park website: http://www.fnal.gov/pub/about/campus/ecology/prairie/index.html.

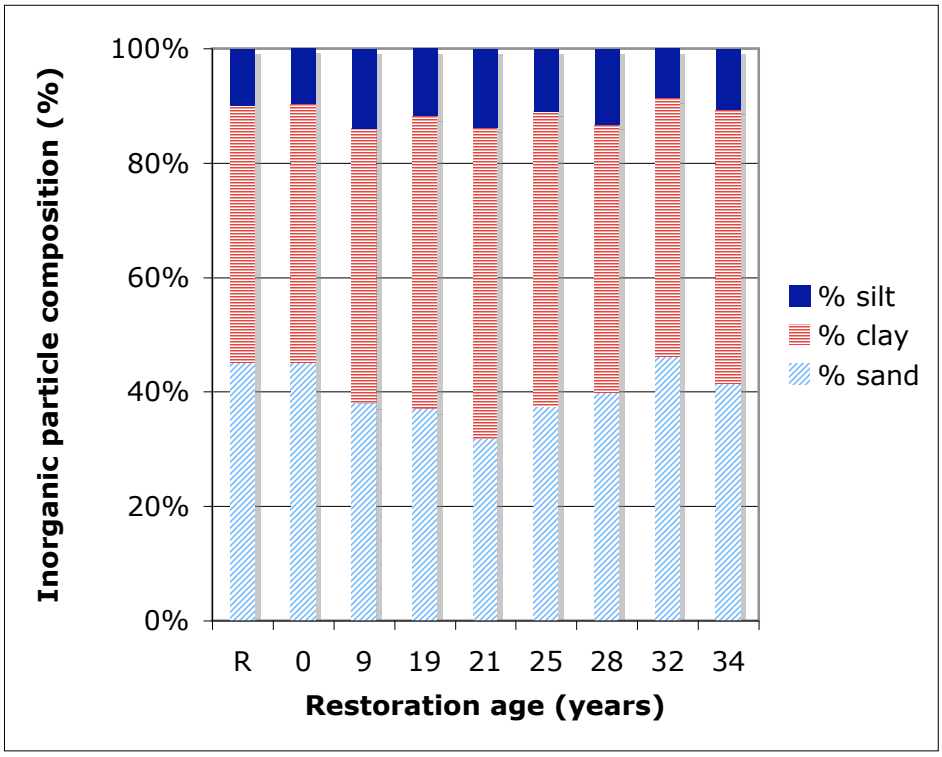

Figure 10. Composition of inorganic soil particles at Fermilab. Note: "R" = Fermilab remnant; "O" = Fermilab agricultural field/baseline value.

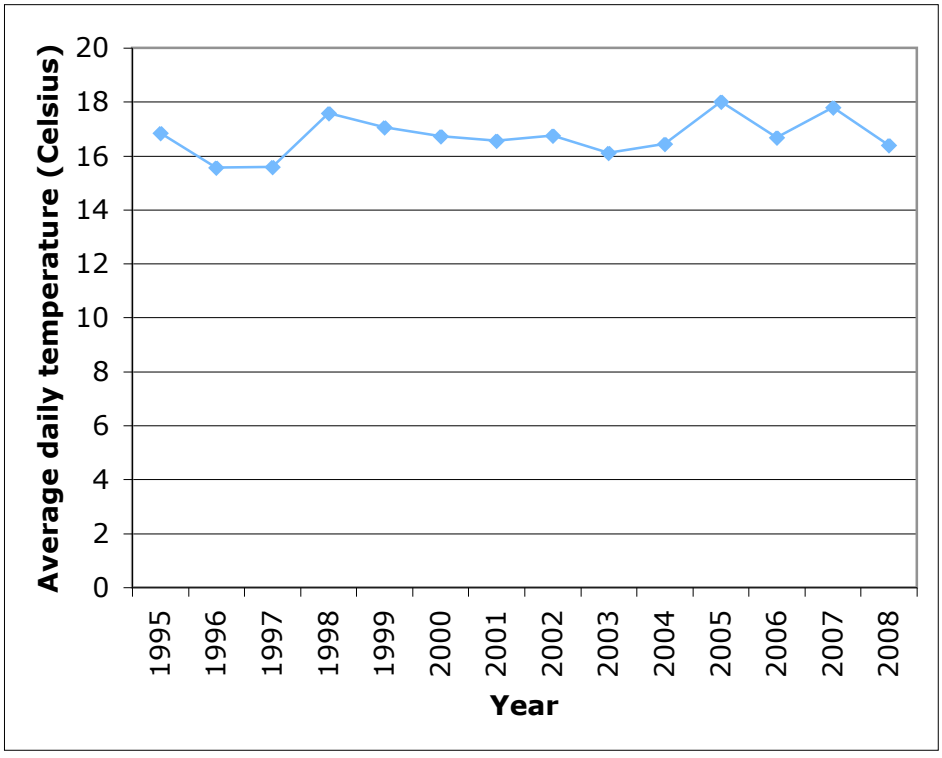

Figure 11. Fermilab average daily temperature during the growing season (April-October).

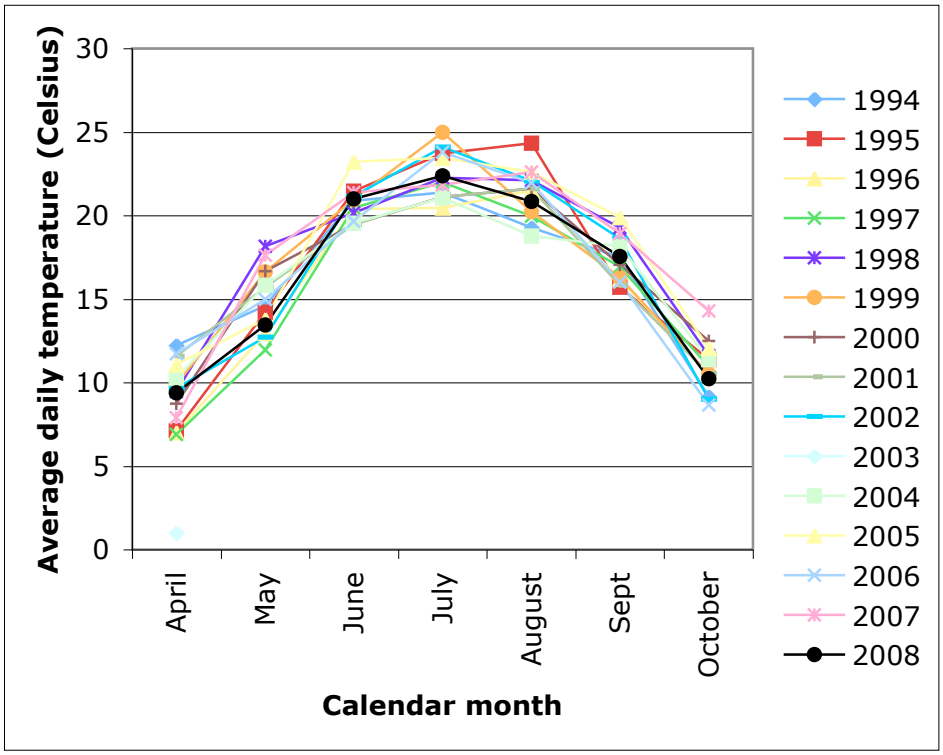

Figure 12. Fermilab average daily temperature from 1994-2008.

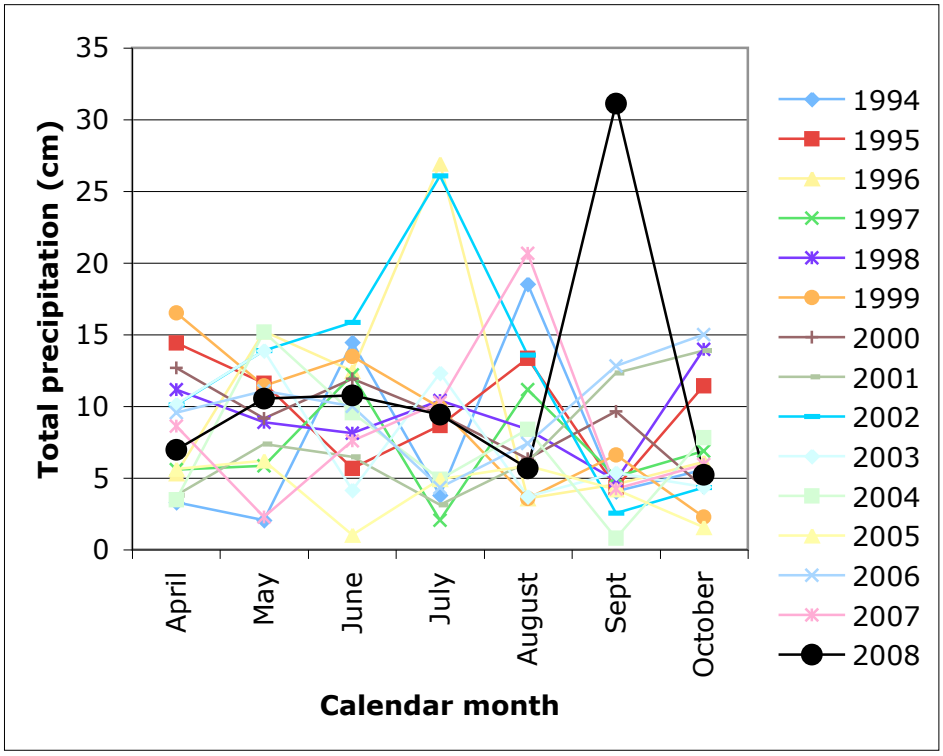

Figure 13. Fermilab monthly total precipitation from 1994-2008.

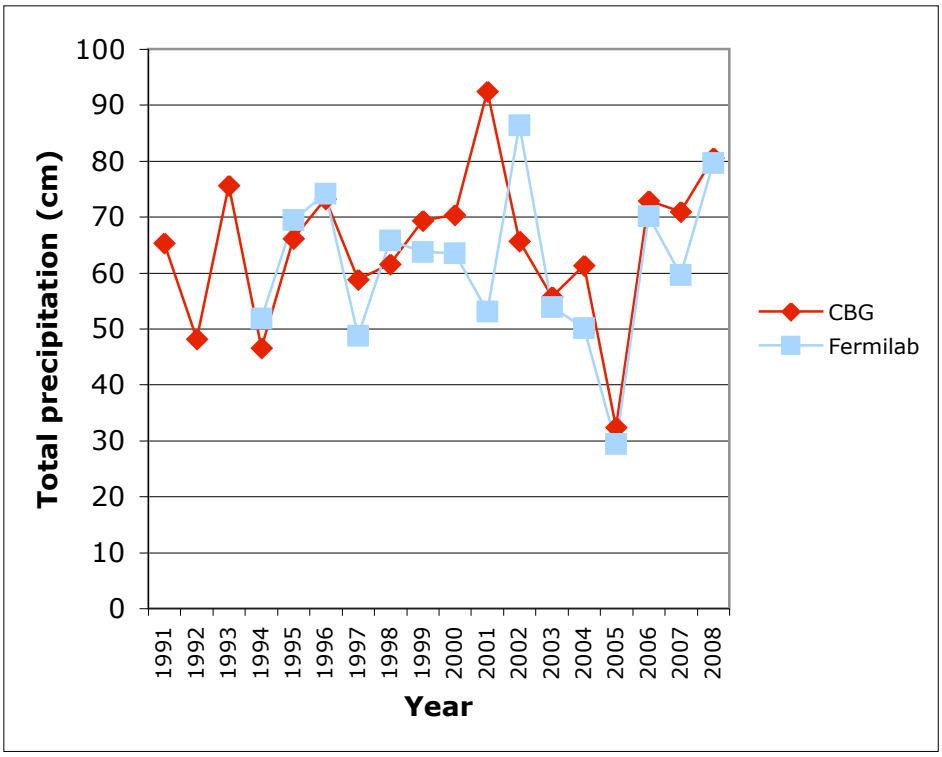

Figure 14. CBG and Fermilab total precipitation during the growing season (April-October).

Soil Analyses[*]

* Unless otherwise noted, no precision/accuracy measurements were made.

Soil core subsample selection

For CBG, soil cores were sliced along the vertical plane, and subsamples were cut off corresponding to the amount needed for a particular test. For Fermilab, in contrast, subsamples were randomly selected from the sampling bag. Due to the difference in selection, the soil at Fermilab may be more representative of the 0-10 cm topsoil layer, whereas at CBG it may be skewed towards a particular vertical plane.

Water-stable Aggregate (WSA) Isolation

WSA were isolated through wet sieving (n= 3/plot Fermilab; n= 2/transect/year CBG), according to the protocol employed by Louise Egerton-Warburton Ph.D. at the Chicago Botanic Garden. 20 g subsamples were placed in a 425 μm sieve and agitated in a cold water bath at ~20 ^{o}C for five minutes. The soil was then gently washed with a stream of water for 10 minutes and oven dried at 75 ^{o}C for 72 hours. Percent soil aggregates present was calculated as follows:

% WSA = 1 – [[(g wet sample corrected for soil moisture) – (g dried aggregates)] / (g wet sample corrected for soil moisture)] x 100

Soil Moisture

10 g of soil (n= 3/ transect/ year CBG; n= 3/plot Fermilab) were weighed and then oven dried at 75 ^{o}C. Percent soil moisture was calculated as follows:

% soil moisture = [(g moist soil)- (g dry soil)] / (g moist soil) x 100

Soil Texture

Soil texture was determined gravimetrically using the hydrometer method (n= 3/ transect/ year CBG; n= 3/plot Fermilab) using the protocol outlined in the Chicago Botanic Garden's REU laboratory manual. 50 g of bulk soil was homogenized with 200 ml of 10% (w/v) sodium hexametaphosphate in a blender until thoroughly dispersed (approximately 30 seconds). The slurry was transferred to a volumetric cylinder and topped with distilled H_2O to the 1000 ml mark. It was then stirred vigorously for 10 seconds and a Fischer brand hydrometer was inserted into the solution. A sand reading was taken at 40 seconds, and afterwards a silt reading was taken at 1 hour 40 minutes.

Inorganic particle size distribution (% sand, silt, clay) was reported via sedimentation rates as related to Stokes law, which posits that the terminal velocity of free-falling particles is related to diameter. Hydrometer readings were corrected with 0.4 g/L added for each degree above 20^{o}C, and 0.4 g/L subtracted for each degree below 20 ^{o}C. The following equations were used to determine percentages of sand, silt, and clay:

% silt + clay = [hydrometer 40 s (g/L) / dry weight of soil sample (g)] * 100

% sand = 100% - (% silt + clay)

% clay= [hydrometer 1 h 40 m (g/L) / dry weight of soil sample (g)] * 100

% silt= (% silt + clay) - % clay

Soil Bulk Density

Soil bulk density was determined using the coated aggregate technique (n= 3/ transect/ year CBG; n= 3/plot Fermilab) using the protocol outlined in the Chicago Botanic Garden's REU laboratory manual. First, a moist soil aggregate weighing approximately 10 g was selected and sealed in Saran wrap. The aggregates were then placed into a plastic beaker filled with water on top of a balance to report mass displacement. The following calculations were used:

Weight of Saran wrap coating (g) = weight of coated aggregate – weight of moisture corrected aggregate

Volume of coating (g/cm^3) = weight of coating / density of Saran wrap ; Density of Saran wrap =1.3 g/cm^3)

Volume of coated aggregate (g/cm^3) = (volume aggregate + coating) – volume of coating

Volume of aggregate (g/cm^3) = (volume of coated aggregate) – (volume of coating)

Bulk density (g/cm^3)= weight of moisture corrected aggregate / volume of aggregate

pH and Conductivity

Soil pH and conductivity were subsequently measured using Hach meters (model unknown) using the protocol employed by Louise Egerton-Warburton Ph.D. at the Chicago Botanic Garden. 10 g of soil (n= 3/ transect/ year CBG; n= 3/plot Fermilab) was placed in 50 ml of distilled H_2O, stirred vigorously, and left to settle for 30 minutes.

Root Biomass

Root biomass was determined through sieving (n= 5 cores/plot Fermilab), or isolation in a cold-water bath (n= 3/transect/year CBG, 10.0 g subsamples). For each method, the root fraction was oven dried at 75 ^{o}C and calculated as follows:

Root biomass (g/g soil) = (g dry roots) / [(g dry roots) + (g moisture corrected soil)]

Microbial Analyses

Mycorrhizal Abundance

Arbuscular mycorrhizal fungi (AMF) colonization was quantified on fine root subsamples (<2 mm). Roots were stained with Trypan blue according to a protocol modified from Koske & Gemma (1989). The roots were loaded into cassettes and cleared in 3% (w/v) potassium hydroxide at 60 ^{o}C for an hour. Cassettes were then rinsed with tap water, soaked in 3% (v/v) hydrogen peroxide at 60 ^{o}C for 20 minutes, and transferred into 1.0% (v/v) hydrochloric acid at 60 ^{o}C for 15 minutes. The samples were then immersed in 0.05% (w/v) Trypan blue stain at 60 ^{o}C for an hour and then de-stained in 50% (v/v) acidic glycerol for approximately 24 hours. Stained roots were mounted onto glass slides using 20% (w/v) polyvinyl alcohol-lactic acid-glycerol mounting solution. AMF structures were observed and calculated

using the line-intersect method at 400x magnification, similar to the protocol outlined in McGonigle et al. (1990). 40 fields of view were scored for hyphae (H), coils (C), vesicles (V), arbuscules (A), and non-colonized, empty fields (N). The proportion of root colonized (T) was calculated as follows:

$$T = (H + V + A + C) / (H + V + A + C + N)$$

Elemental Analyses

Soil aggregate % C and N

500 mg of soil (n= 3/plot Fermilab, n= 7/year CBG) was pulverized to powder using a bead beater (Biopsec Products model no. 3110). Samples were analyzed at the Kansas State Soil Agronomy Laboratory using a LECO CN 2000 combustion analyzer. The results were reported on a weight percent (%) basis. The precision for carbon was 25 parts per million (ppm), whereas for nitrogen it was 40 ppm.

Root % C and N

Fine root matter (<2 mm diameter) (n=1/year CBG; n=1/ plot Fermilab) was prepared using the protocol employed by Louise Egerton-Warburton Ph.D. at the Chicago Botanic Garden. Bulk root matter was washed free of adhering soil particles using a cold-water bath for 5 minutes, rinsing with distilled water, removing any soil impurities with tweezers, and by rinsing again. The samples were oven-dried at 75^{o}C for 48 hours, and fine root matter was pulverized using a bead beater. 200 mg fine root subsamples were analyzed (n= 1 per year CBG; n= 1 per site Fermilab; C3 and C4 positive controls n= 3/species/site). The precision for carbon was 25 parts per million (ppm), whereas for nitrogen it was 40 ppm.

RESULTS

Statistical Analyses

Statistical analyses were conducted using Stata 11 (StataCorp, 2009). Specifically, regression analysis and/or analysis of variance (ANOVA) were employed. Interactive effects were tested in respect to site to determine if the coefficient of the Ordinary Least Squares (OLS) estimators were significantly different. Data were corrected for heteroskedasticity, but were not log transformed due to the constraints imposed by dissimilar sampling methodologies and low sample size. 95% confidence intervals were used to denote statistical significance. Descriptive statistics for the various soil parameters are presented in Tables 34-42, and raw data are reported in Appendix A.

Soil macroaggregate abundance exhibited a statistically significant increase at Fermilab ($p<0.001$), whereas at CBG there was a decrease over the period of interest ($p<0.05$) (Figure 19; Tables 1-2). A significant interactive effect was reported for site in the relationship between macroaggregate abundance and restoration age ($p< 0.001$) (Table 3). At Fermilab, the 34-year-old restoration plot reported a macroaggregate abundance of 58.95% ($\sigma = 6.03\%$) verses 73.13% ($\sigma = 2.74\%$) at the remnant site. At CBG, 26 years post-prairie creation macroaggregate abundance was 13.51% ($\sigma = 3.51\%$), down from the highest point recorded 15 years post-prairie creation at 35.45% ($\sigma =5.21\%$).

Soil macroaggregate carbon concentration (% carbon by weight) increased at each site, but was not significant at the $p<0.05$ level (Figure 20; Tables 4-5). No significant interactive effect was reported in respect to site (Table 6). Carbon concentration increased at roughly the same rate, but was approximately 1.96% higher at CBG during the period of interest. Currently,

the 32-year-old restoration plot at Fermilab reported an average carbon concentration similar to the remnant site (x= 6.43%, σ = 0.47% verses x= 6.42%, σ = 0.08%, respectively). At CBG, the average carbon concentration increased from 6.83% (σ = 1.46%) to 7.51% (σ = 1.18%) for the period between 9 to 26 years. Soil macroaggregate carbon:nitrogen (C:N) concentration was not statistically significant at either site (Figure 21; Tables 7-8), and there was no evidence of an interactive effect (Table 9).

Soil macroaggregate carbon storage exhibited a statistically significant increase with age at Fermilab ($p<0.001$), but decreased at CBG over the period of study ($p<0.01$) (Figure 22; Tables 10-11). A significant interactive effect was reported in respect to site ($p<0.001$) (Table 12). At Fermilab, macroaggregate carbon storage increased from 1.51% (σ = 0.05%) in the agricultural field up to 3.84% (σ= 0.21%) in the 32-year-old restoration plot. At CBG, carbon storage decreased from 1.46% (σ = 0.29%) to 1.02% (σ = 0.16%) for the time period between 9 to 26 years post-prairie creation. At 15 years post-prairie creation, the average macroaggregate carbon concentration per unit soil was 2.82% (σ = 0.40%), higher than any other point reported over the period of interest. The results are highly concomitant with macroaggregate abundance.

Arbuscular mycorrhizal fungi (AMF) root colonization exhibited a statistically significant increase at Fermilab ($p<0.01$), but a decrease at CBG that was not significant at the $p<0.05$ level (Figure 23; Tables 13-14). A significant interactive effect was reported in respect to site ($p<0.001$) (Table 15). At Fermilab, during the period between 9 and 34 years post-restoration, AMF colonization increased from 75.62% (σ = 4.59%) to 94.80% (σ = 0.66%). Interestingly, AMF colonization in the remnant plot was 70.89% (σ = 3.30%), lower than any other value. At CBG, AMF colonization decreased from 77.82% (σ = 1.47%) to 50.25% (σ = 15.52%) between

9 to 26 years post-prairie creation. Root colonization reported the largest value at 15 years post-restoration with 82.42% colonization (σ = N/A; n=1).

Fine root C:N exhibited a decrease at Fermilab, but was not significant at the $p<0.05$ level (Figure 24; Table 16). At CBG, there was no discernable trend (Figure 24; Table 17), and no interactive effect was supported in respect to site (Table 18). Fine root controls for C3 and C4 species yielded no significant difference when compared across sites (Table 19) or within sites (Tables 20-21).

Bulk root biomass exhibited a statistically significant decrease with age at CBG ($p<0.01$), but there was no discernable trend at Fermilab (Figure 25; Tables 22-23). There was no evidence for an interactive effect (Table 24). However, when considering the differences in sampling methodology and low statistical power, it was determined that the data was not reliable.

Soil bulk density exhibited a statistically significant increase at Fermilab ($p<0.001$), but there was a significant decrease at CBG ($p<0.001$) (Figure 26; Tables 25-26). A significant interactive effect was reported in respect to site ($p<0.001$) (Table 27). At Fermilab, bulk density decreased from 0.85 g/cm^3 (σ = 0.002) in the 9-year-old plot to 0.82 g/cm^3 (σ= 0.002) in the 34-year-old plot. Remnant conditions have been attained in the 32-year-old and 34-year-old plots. In contrast, at CBG, bulk density increased from 0.71 g/cm^3 (σ = 0.013) to 0.75 g/cm^3 (σ = 0.002) during 9 to 26 years post-prairie creation.

Soil bulk pH was not statistically significant at either site (Figure 27; Tables 28-29), and there was no evidence of a significant interactive effect (Table 30). Bulk soil electrical conductivity (EC) exhibited a statistically significant decrease at CBG ($p<0.001$), but no discernable trend was found at Fermilab (Tables 28; Tables 31-32). A significant interactive

effect was reported in respect to site ($p<0.001$) (Table 33). At CBG, EC decreased from 168.89 µS ($\sigma = 19.74$) to 74.67 µS ($\sigma = 5.68$) during 9 to 26 years post-prairie creation.

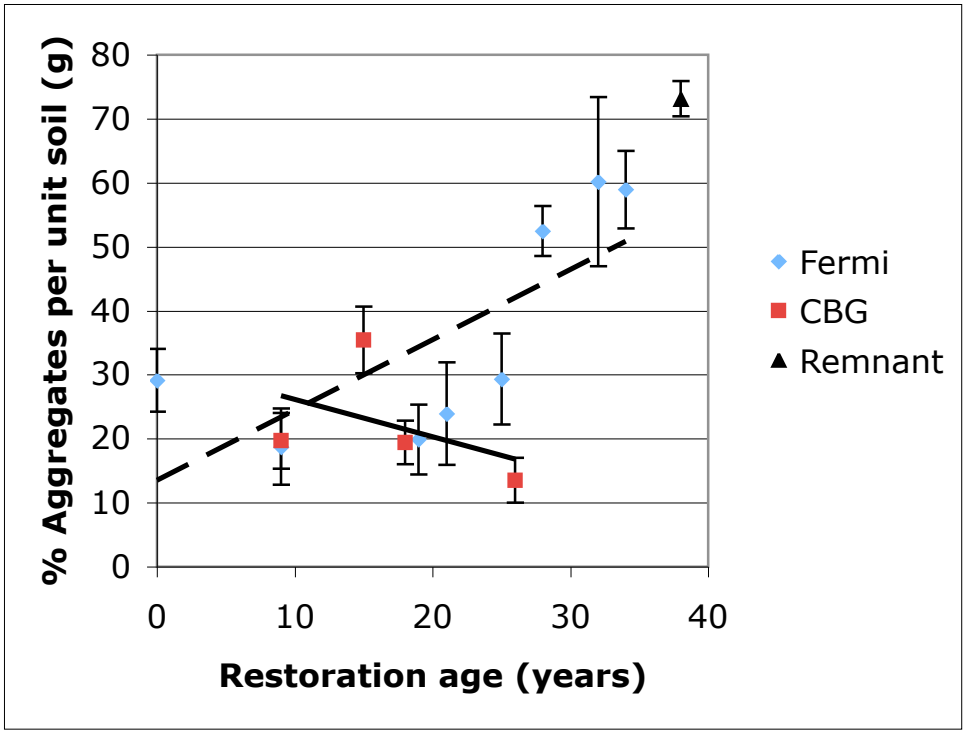

Figure 15. Average soil aggregate abundance (%) verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.58	0.23	-2.53	0.019	-1.06	-0.11
Constant	31.93	5.02	6.36	0.000	21.52	42.35
Y= -0.58x+31.93; r^2= 0.15776; n= 24						

Table 1. CBG linear regression model for soil water-stable aggregate abundance (% aggregates/bulk soil (g)).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	1.10	0.27	4.12	0.000	0.54	1.65
Constant	13.54	6.44	2.10	0.047	0.19	26.89
Y= 1.10x+13.54; r^2= 0.4622; n= 24						

Table 2. Fermilab linear regression model for soil water-stable aggregate abundance (% aggregates/bulk soil (g)).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.58	0.23	-2.53	0.015	-1.05	-0.12
Site	-18.39	8.17	-2.25	0.029	-34.85	-1.93
Age x Site	1.68	0.35	4.77	0.000	0.97	2.39
Constant	31.93	5.02	6.36	0.000	21.81	42.05
r^2= 0.5276, n= 48						

Table 3. Interaction table for soil water-stable aggregate abundance (% aggregates/bulk soil (g)). Note: CBG was used as the dummy (indicator) variable for the table.

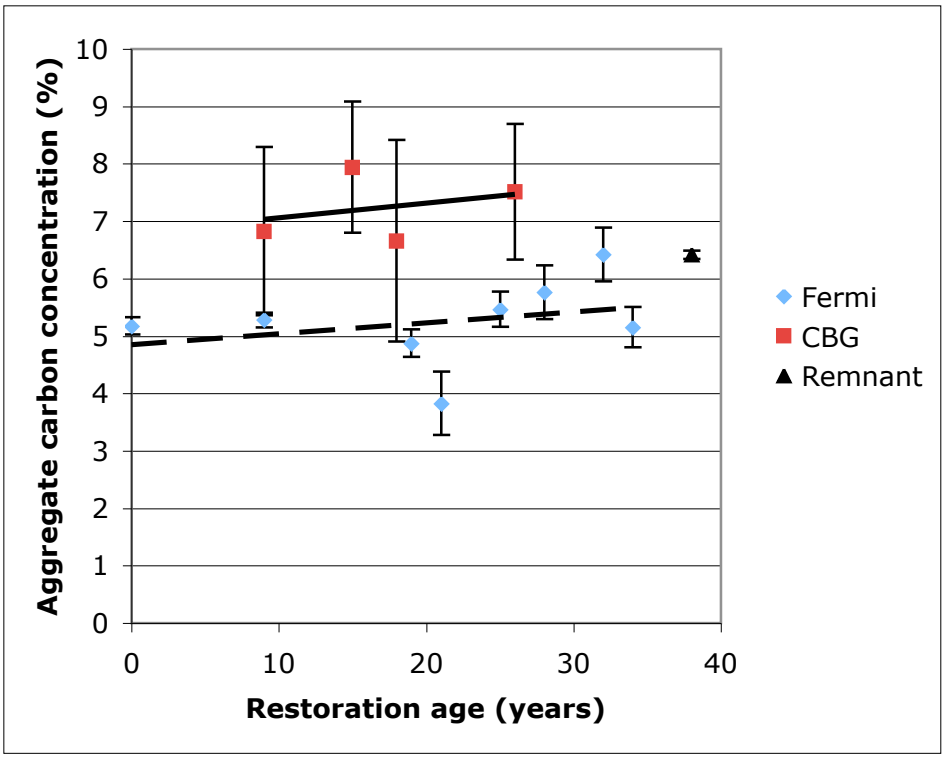

Figure 16. Average soil aggregate carbon concentration (% / weight) verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.03	0.04	0.64	0.526	-0.06	0.11
Constant	6.80	0.75	9.05	0.000	5.26	8.35
Y= 0.03x+6.80; r^2= 0.0122; n= 28						

Table 4. CBG linear regression model for soil water-stable aggregate carbon concentration (% / weight).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.02	0.01	1.90	0.070	0.00	0.04
Constant	4.84	0.21	23.47	0.000	4.42	5.27
Y= 0.02x+4.84; r^2= 0.0757; n= 24						

Table 5. Fermilab linear regression model for soil water-stable aggregate carbon concentration (% / weight).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.03	0.04	0.64	0.524	-0.05	0.10
Site	-1.96	0.78	-2.51	0.016	-3.53	-0.39
Age x Site	-0.01	0.04	-0.15	0.880	-0.09	0.08
Constant	6.80	0.75	9.03	0.000	5.29	8.32
r^2= 0.4401; n= 52						

Table 6. Interaction table for soil water-stable aggregate carbon concentration (% / weight). Note: Site was used as the dummy (indicator) variable for the table.

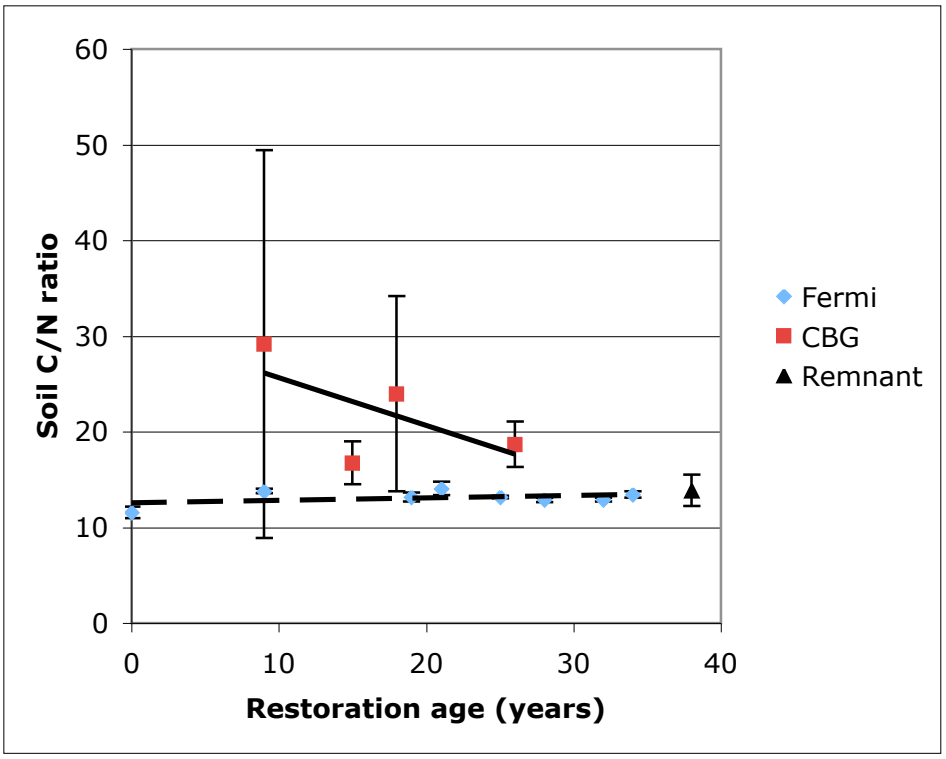

Figure 17. Average soil aggregate C:N ratio verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.50	0.40	-1.23	0.229	-1.33	0.33
Constant	30.61	8.76	3.49	0.002	12.59	48.62
Y= -0.50x+30.61; r^2= 0.0684; n= 28						

Table 7. CBG linear regression model for soil water-stable aggregate C:N ratio.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.03	0.02	1.48	0.152	-0.01	0.06
Constant	12.57	0.48	26.13	0.000	11.57	13.57
Y= 0.03x+12.57; r^2= 0.1317; n= 24						

Table 8. Fermilab linear regression model for soil water-stable aggregate C:N ratio.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.50	0.41	-1.23	0.225	-1.31	0.32
Site	-18.03	8.80	-2.05	0.046	-35.73	-0.33
Age x Site	0.52	0.41	1.29	0.202	-0.29	1.34
Constant	30.61	8.79	3.48	0.001	12.93	48.28
r^2=0.2691; n=52						

Table 9. Interaction table for soil water-stable aggregate C:N ratio. Note: Site was used as the dummy (indicator) variable for the table.

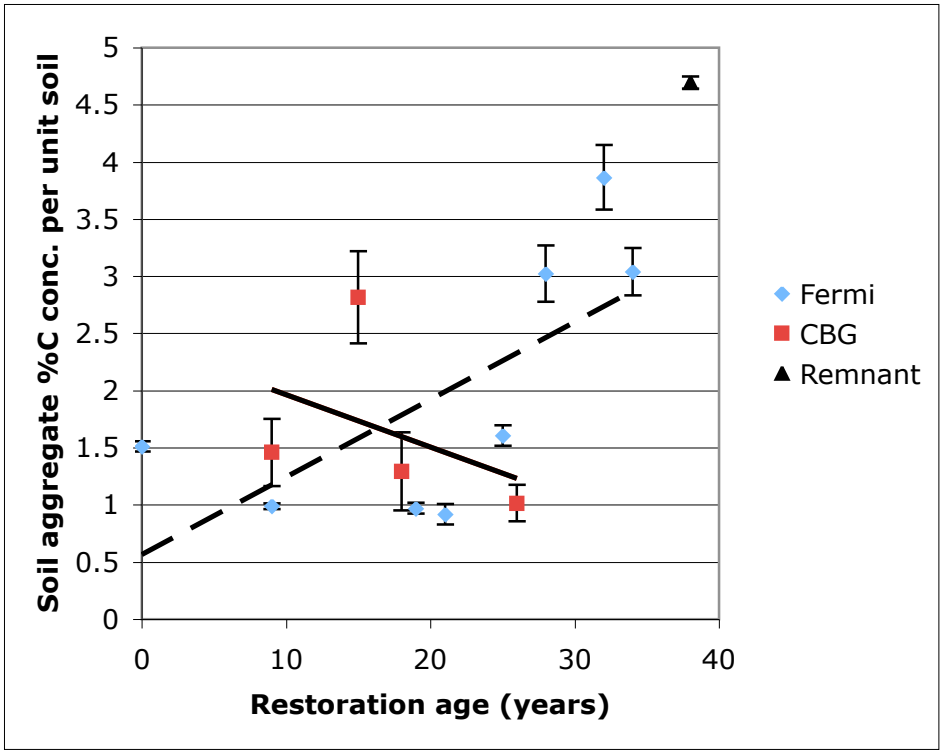

Figure 18. Average soil aggregate %C per unit soil verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.05	0.02	-2.95	0.007	-0.08	-0.01
Constant	2.43	0.36	6.71	0.000	1.68	3.17
Y= -0.05x+2.43; r^2= 0.1395; n= 28						

Table 10. CBG linear regression model for soil water-stable aggregate carbon (% per unit soil).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.07	0.02	4.35	0.000	0.04	0.10
Constant	0.56	0.37	1.51	0.144	-0.21	1.34
Y= 0.07x+0.56; r^2= 0.4629; n= 24						

Table 11. Fermilab linear regression model for soil water-stable aggregate carbon (% per unit soil).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.05	0.02	-2.94	0.005	-0.08	-0.01
Site	-1.86	0.52	-3.59	0.001	-2.90	-0.82
Age x Site	0.11	0.02	5.16	0.000	0.07	0.16
Constant	2.43	0.36	6.69	0.000	1.70	3.15
r^2= 0.3686; n= 52						

Table 12. Interaction table for soil water-stable aggregate carbon (% per unit soil).Note: Site was used as the dummy (indicator) variable for the table.

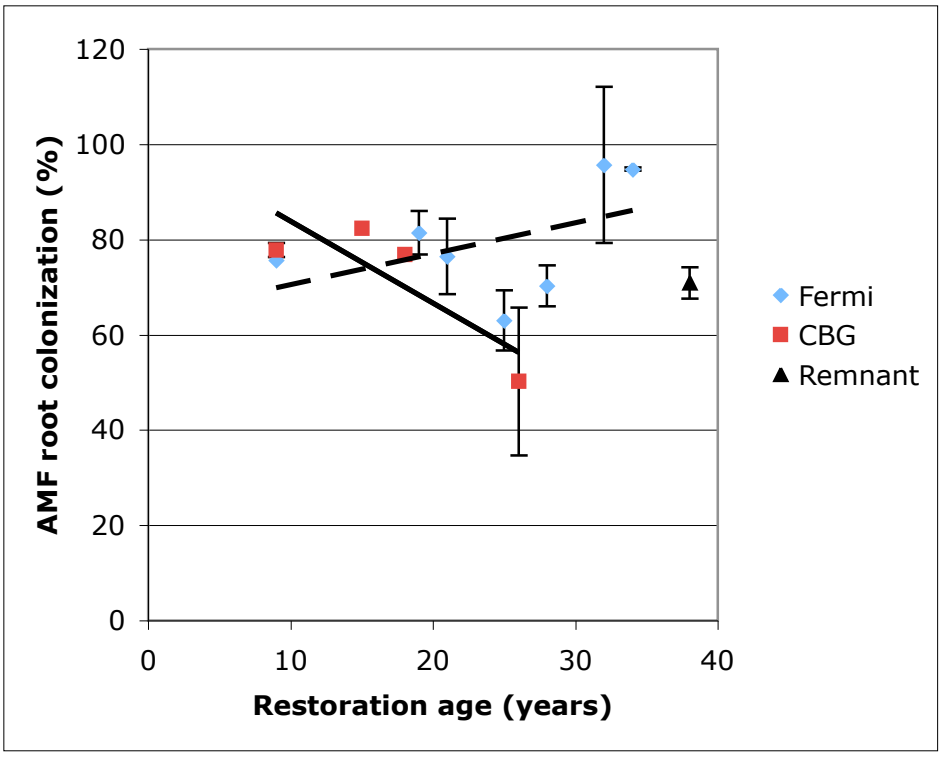

Figure 19. Average arbuscular mycorrhizal fungi root colonization verses restoration age. Note: Data points without error bars have a sample size of n=1.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-1.70	0.78	-2.18	0.095	-3.87	0.47
Constant	98.52	10.97	8.98	0.001	68.07	128.97
Y= -1.70x+98.52; r^2= 0.5773; n= 6						

Table 13. CBG linear regression model for arbuscular mycorrhizal fungi root colonization (%).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	1.04	0.32	3.20	0.008	0.33	1.74
Constant	55.71	8.33	6.69	0.000	37.56	73.85
Y= 1.04x+55.71; r^2= 0.4446; n= 14						

Table 14. Fermilab linear regression model for arbuscular mycorrhizal fungi root colonization (%).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-1.70	0.71	-2.39	0.030	-3.21	-0.19
Site	-42.81	13.21	-3.24	0.005	-70.82	-14.81
Age x Site	2.74	0.79	3.48	0.003	1.07	4.41
Constant	98.52	10.01	9.84	0.000	77.30	119.74
r^2= 0.5232; n=20						

Table 15. Interaction table for arbuscular mycorrhizal fungi root colonization (%). Note: Site was used as the dummy (indicator) variable for the table.

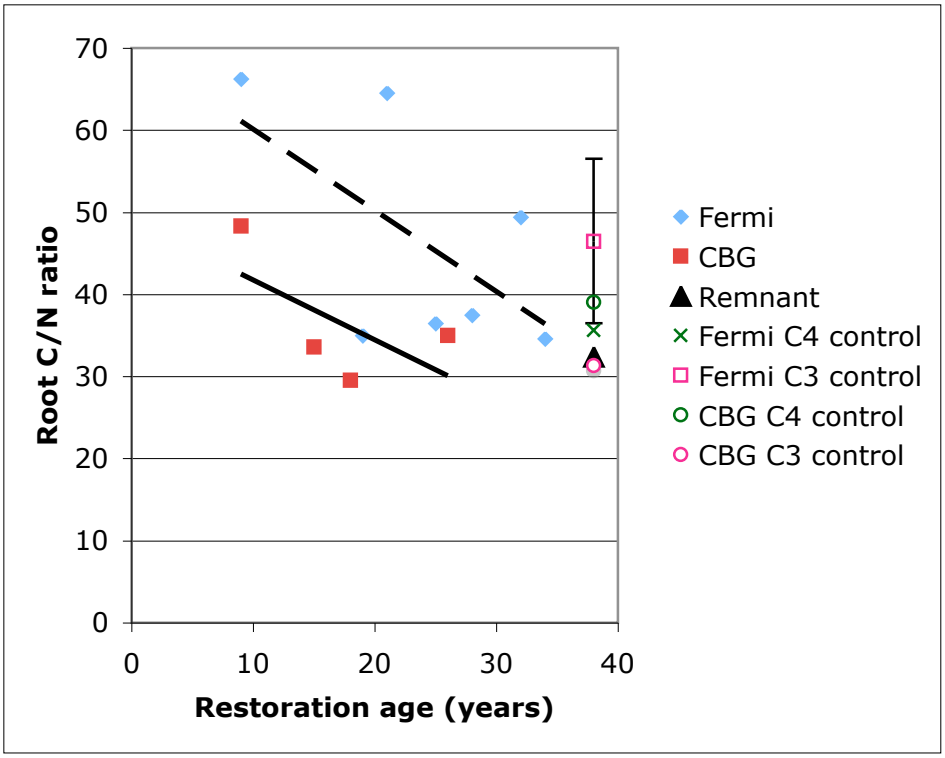

Figure 20. Average fine root (<2 mm) C:N verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.73	0.62	-1.18	0.361	-3.40	1.94
Constant	49.03	11.51	4.26	0.051	-0.51	98.57
Y= -0.73x+49.03; r^2= 0.3999; n= 4						

Table 16. CBG linear regression model for fine root (< 2 mm) C:N.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.99	0.41	-2.41	0.061	-2.04	0.07
Constant	69.99	12.06	5.81	0.002	39.00	100.99
Y= -0.99x+69.99; r^2= 0.3650; n= 7						

Table 17. Fermilab linear regression model for fine root (< 2 mm) C:N.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.73	0.55	-1.33	0.226	-2.03	0.57
Site	20.96	16.35	1.28	0.241	-17.70	59.62
Age x Site	-0.26	0.70	-0.37	0.722	-1.92	1.40
Constant	49.03	10.21	4.80	0.002	24.90	73.16
r^2= 0.4622; n= 11						

Table 18. Interaction table for fine root (< 2 mm) C:N. Note: Site was used as the dummy (indicator) variable for the table.

Group	Obs.	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
C3	6	38.90	5.23	12.81	25.46	52.34
C4	6	37.37	3.88	9.50	27.40	47.35
Combined	12	38.14	3.11	10.78	31.29	44.99
Difference		1.53	6.51		-13.15	16.20
p-value= 0.8198						

Table 19. ANOVA table for difference in C3 and C4 positive root controls (pooled for site).

Group	Obs.	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
CBG	3	39.09	1.80	3.12	31.33	46.85
Fermi	3	35.66	8.31	14.39	-0.10	71.41
Combined	6	37.37	3.88	9.50	27.40	47.35
Difference		3.44	8.50		-20.17	27.04
p-value= 0.7069						

Table 20. ANOVA table for C4 positive root controls between sites.

Group	Obs.	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
CBG	3	31.31	5.01	8.68	9.74	52.88
Fermi	3	46.49	7.35	12.73	14.88	78.11
Combined	6	38.90	5.23	12.81	25.46	52.34
Difference		-15.19	8.89		-39.88	9.51
p-value= 0.1630						

Table 21. ANOVA table for C3 positive root controls between sites.

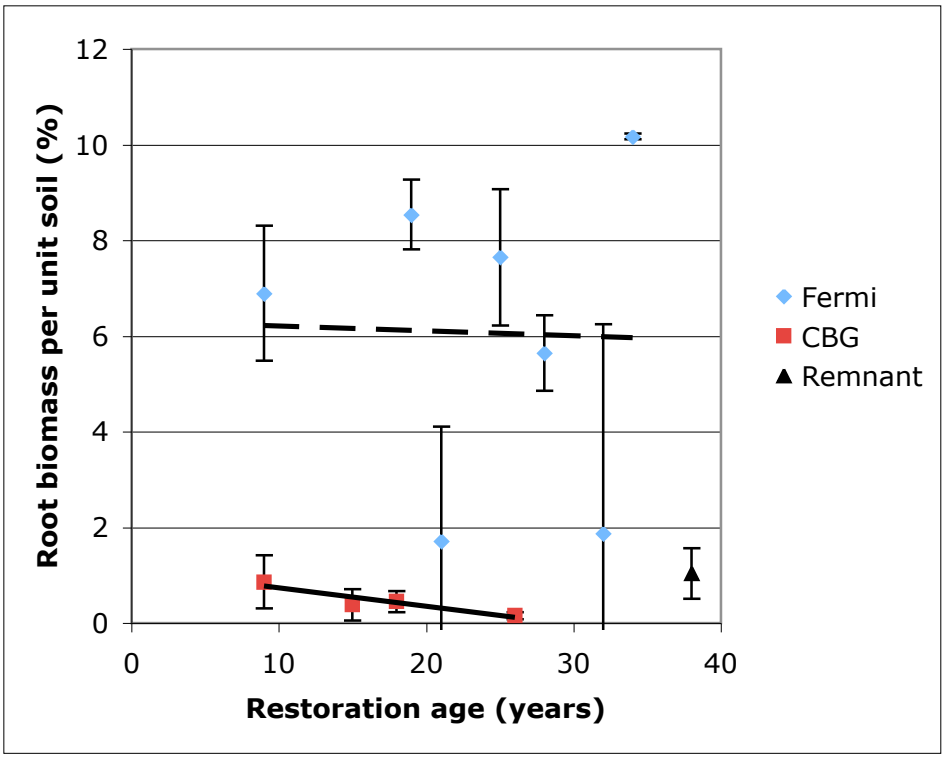

Figure 21. Average bulk root biomass verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.04	0.01	-3.21	0.004	-0.06	-0.01
Constant	1.12	0.26	4.25	0.000	0.57	1.66
Y= -0.04x+1.12; r^2= 0.3433; n= 24						

Table 22. CBG linear regression model for bulk root biomass (%).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.00	0.10	-0.04	0.971	-0.20	0.20
Constant	6.09	2.33	2.62	0.014	1.34	10.85
Y= 0x+6.09; r^2= 0.0001; n= 33						

Table 23. Fermilab linear regression model for bulk root biomass (%).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.04	0.01	-3.24	0.002	-0.06	-0.01
Site	4.98	2.36	2.11	0.039	0.25	9.70
Age x Site	0.03	0.10	0.35	0.725	-0.16	0.23
Constant	1.12	0.26	4.28	0.000	0.59	1.64
r^2= 0.4602; n= 57						

Table 24. Interaction table for bulk root biomass (%). Note: Site was used as the dummy (indicator) variable for the table.

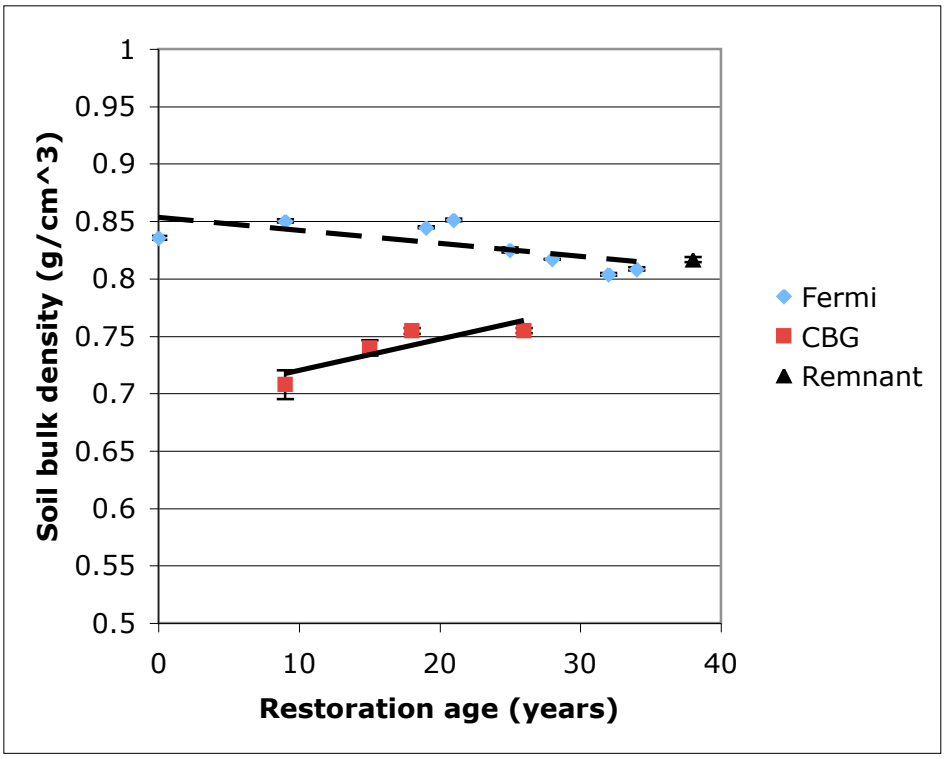

Figure 22. Average soil bulk density verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.0028	0.0004	6.95	0.000	0.0020	0.0036
Constant	0.6916	0.0077	89.54	0.000	0.6756	0.7076
Y= 0.0028x+0.6916; r^2= 0.6777; n= 25						

Table 25. CBG linear regression model for soil bulk density (g/cm^3).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.0011	0.0003	-4.22	0.000	-0.0017	-0.0006
Constant	0.8533	0.0070	121.91	0.000	0.8388	0.8678
Y= -0.0011x+0.8533; r^2= 0.4938; n= 24						

Table 26. Fermilab linear regression model for soil bulk density (g/cm^3).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.0028	0.0004	6.94	0.000	0.0020	0.0036
Site	0.1617	0.0104	15.51	0.000	0.1407	0.1827
Age x Site	-0.0039	0.0005	-8.12	0.000	-0.0049	-0.0029
Constant	0.6916	0.0077	89.46	0.000	0.6760	0.7072
r^2= 0.9400; n=49						

Table 27. Interaction table for soil bulk density (g/cm^3). Note: Site was used as the dummy (indicator) variable for the table.

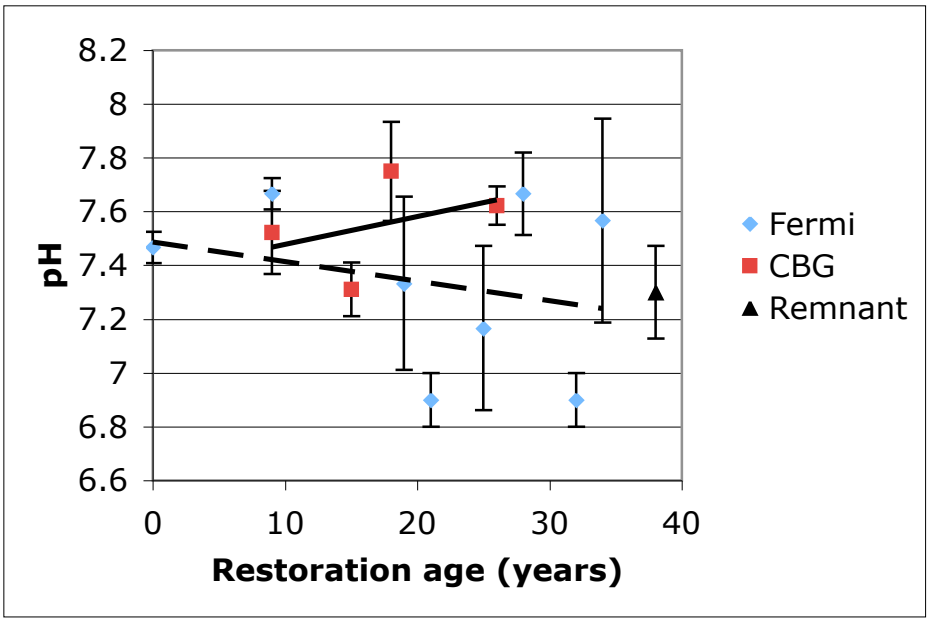

Figure 23. Average bulk soil pH verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.01	0.01	1.43	0.164	-0.01	0.03
Constant	7.37	0.21	34.56	0.000	6.93	7.81
Y= 0.01+7.37; r^2= 0.0363; n= 28						

Table 28. CBG linear regression model for bulk soil pH.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-0.01	0.01	-1.36	0.187	-0.02	0.00
Constant	7.49	0.09	81.51	0.000	7.30	7.68
Y= -0.01x+7.49; r^2= 0.0522; n= 24						

Table 29. Fermilab linear regression model for bulk soil pH.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.01	0.01	1.43	0.160	-0.01	0.03
Site	0.12	0.23	0.50	0.618	-0.35	0.58
Age x Site	-0.02	0.01	-1.90	0.063	-0.04	0.00
Constant	7.37	0.21	34.45	0.000	6.94	7.80
r^2= 0.1398; n= 52						

Table 30. Interaction table for bulk soil pH. Note: Site was used as the dummy (indicator) variable for the table.

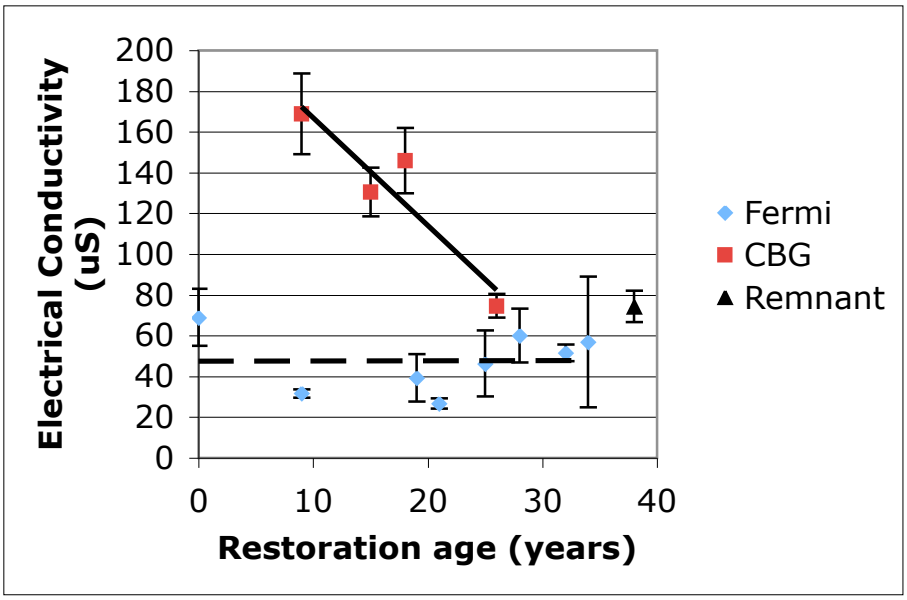

Figure 24. Average bulk soil Electrical Conductivity verses restoration age.

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-5.46	1.03	-5.29	0.000	-7.58	-3.33
Constant	216.84	21.69	10.00	0.000	172.26	261.43
Y= -5.46x+216.84; r^2= 0.4585; n= 28						

Table 31. Fermilab linear regression model for bulk soil Electrical Conductivity (µS).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	0.01	0.43	0.02	0.986	-0.88	0.89
Constant	47.55	10.09	4.71	0.000	26.63	68.47
Y= 0.01+47.55; r^2= 0.0000; n= 24						

Table 32. Fermilab linear regression model for bulk soil Electrical Conductivity (µS).

	Coef.	Std. Err.	t-statistic	P > t	[95% Confidence Int.]	
Age	-5.46	1.04	-5.27	0.000	-7.54	-3.37
Site	-169.29	23.97	-7.06	0.000	-217.48	-121.10
Age x Site	5.46	1.12	4.88	0.000	3.21	7.72
Constant	216.84	21.76	9.97	0.000	173.10	260.58
r^2=0.7031, n= 52						

Table 33. Interaction table for bulk soil Electrical Conductivity (µS). Note: Site was used as the dummy (indicator) variable for the table.

Site	Restoration age (years)	Soil aggregation (%)	σ
CBG	9	19.69	4.37
	15	35.45	5.21
	18	19.43	3.40
	26	13.51	3.51
Fermi Ag Field	0	29.15	4.88
Fermi	9	18.75	5.96
	19	19.88	5.48
	21	23.94	8.01
	25	29.36	7.08
	28	52.44	3.92
	32	60.14	13.22
	34	58.95	6.03
Fermi Remnant	>150	73.13	2.75

Table 34. Descriptive statistics for soil aggregate abundance data.

Site	Restoration age (years)	Soil Aggregate %C	σ	Soil Aggregate C/N ratio	σ
CBG	9	6.83	1.46	29.16	20.28
	15	7.94	1.14	16.75	2.23
	18	6.66	1.76	23.96	10.21
	26	7.51	1.18	18.68	2.36
Fermi Ag	0	5.18	0.15	11.56	0.60
Fermi	9	5.28	0.13	13.81	0.26
	19	4.88	0.24	13.18	0.46
	21	3.83	0.55	14.07	0.71
	25	5.47	0.30	13.16	0.13
	28	5.77	0.47	12.88	0.24
	32	6.43	0.47	12.88	0.20
	34	5.16	0.35	13.47	0.33
Fermi Remnant	>150	6.42	0.08	13.87	1.64

Table 35. Descriptive statistics for soil aggregate elemental analysis data at each site.

Site	Restoration age (years)	Soil Aggregate %C / unit soil	σ
CBG	9	1.46	0.29
	15	2.82	0.40
	18	1.29	0.34
	26	1.02	0.16
Fermi Ag	0	1.51	0.04
Fermi	9	0.99	0.02
	19	0.97	0.05
	21	0.92	0.09
	25	1.60	0.09
	28	3.02	0.24
	32	3.86	0.28
	34	3.04	0.21
Fermi Remnant	>150	4.70	0.06

Table 36. Descriptive statistics for soil aggregate % carbon per unit soil.

Site	Restoration age (years)	AMF % root colonization	σ
CBG	9	77.82	1.47
	15	82.42	N/A
	18	76.92	N/A
	26	50.25	15.52
Fermi Ag	0	41.82	N/A
Fermi	9	75.62	4.59
	19	81.46	7.91
	21	76.42	6.35
	25	63.05	4.31
	28	70.32	16.39
	32	95.70	0.39
	34	94.80	0.66
Fermi Remnant	>150	70.89	3.30

Table 37. Descriptive statistics for arbuscular mycorrhizal root colonization data. Note: The "N/A" entries had sample size n=1.

Site	Restoration age (years)	Total %C	Total %N	C/N ratio
CBG	9	44.81	0.93	48.36
	15	39.45	1.17	33.61
	18	40.15	1.36	29.53
	26	42.13	1.20	35.01
Fermi	9	43.22	0.65	66.28
	19	37.41	1.07	35.02
	21	39.60	0.61	64.54
	25	37.81	1.04	36.48
	28	38.72	1.03	37.51
	32	41.29	0.84	49.40
	34	32.38	0.94	34.60
Fermi Remnant	>150	41.83	1.29	32.42

Table 38. Fine root (<2 mm diameter) elemental analysis. Note: Sample size is n=1.

Site	Control	C/N ratio	σ
CBG	C3	31.31	8.68
	C4	39.09	3.12
Fermi	C3	46.49	12.73
	C4	35.66	14.39

Table 39. Descriptive statistics for fine root (<2 mm diameter) elemental analysis for C3 and C4 control (CBG: C3= *Solidago altissima*, C4= *Spartina pectinata*; Fermilab: C3= *Solidago altissima*, C4= *Sorghastrum nutans*).

Site	Restoration age (years)	Root biomass (%)	σ
CBG	9	0.86	0.55
	15	0.38	0.33
	18	0.45	0.23
	26	0.16	0.07
Fermi Ag Field	0	0.09	0.06
Fermi	9	6.89	4.34
	19	8.54	1.42
	21	1.72	0.73
	25	7.65	2.38
	28	5.64	1.42
	32	1.87	0.79
	34	10.17	4.38
Fermi Remnant	>150	1.04	0.53

Table 40. Descriptive statistics for bulk root biomass data.

Site	Restoration age (years)	Avg. bulk density (g/cm^3)	σ
CBG	9	0.71	0.013
	15	0.74	0.007
	18	0.75	0.003
	26	0.75	0.002
Fermi Ag Field	0	0.84	0.002
Fermi	9	0.85	0.002
	19	0.84	0.001
	21	0.85	0.001
	25	0.82	0.002
	28	0.82	0.000
	32	0.80	0.001
	34	0.81	0.001
Fermi Remnant	>150	0.82	0.002

Table 41. Descriptive statistics for soil bulk density data.

Site	Restoration age (years)	pH	σ	EC (µS)	σ
CBG	9	7.52	0.16	168.89	19.74
	15	7.31	0.10	130.56	11.88
	18	7.75	0.18	146.00	16.02
	26	7.62	0.07	74.67	5.68
Fermi Ag Field	0	7.47	0.06	69.00	14.00
Fermi	9	7.67	0.06	31.67	2.08
	19	7.33	0.32	39.33	11.68
	21	6.90	0.10	26.67	2.52
	25	7.16	0.30	46.33	16.20
	28	7.67	0.15	60.00	13.23
	32	6.90	0.10	51.67	4.04
	34	7.57	0.38	57.00	32.08
Fermi Remnant	>150	7.30	0.17	74.33	7.64

Table 42. Descriptive statistics for bulk soil pH and Electrical Conductivity (EC) data.

DISCUSSION

Overview of the Current Study

The current study compared soil macroaggregate carbon storage (>425 μm) at an artificially created prairie site located on marshland soil with a formerly cultivated site located on native prairie soil. Specifically, the comparative analysis attempted to develop a better understanding about how soil origin and site history influences below-ground succession by measuring the factors associated with soil aggregate formation. The water-stable macroaggregate soil fraction was isolated because it is water-insoluble and relatively resistant to erosion, leaching, and microbial oxidation (Tisdall & Oades, 1982). Also, macroaggregates are bound together by microbial and root exudates, so it is an indirect reflection of the propensity of soil particles to adhere to each other (Wright & Upadhyaya, 1998). Although the majority of research has focused on the 250 μm fraction, investigating a larger size class, 425 μm in this case, might be more relevant to sustainable carbon storage because a positive correlation has been observed between aggregate size and carbon concentration (Angers & Giroux, 1996; Jastrow et al., 1996). At both sites, soil macroaggregate carbon was hypothesized to increase with restoration age as a result of SOM depletion incurred prior to restoration.

For the purpose of this study, the Chicago Botanic Garden Suzanne S. Dixon Prairie (CBG) was defined as an "artificially created landscape." Constructed on marshland in the 1960s, bulldozers and other heavy machinery were used to excavate and to sculpt the terrain. Select areas contain dredged material and, prior to the prairie being established in 1982, approximately 6 inches of imported topsoil was applied (D. Sollenberger, pers. comm.). At Fermilab, the formerly cultivated prairie restoration is located on native prairie soil, and multiple

lines of evidence indicate an ecological trajectory towards remnant prairie conditions (Jastrow, 1987; Miller & Jastrow 1990; Betz, 1995; Jastrow et al., 1998; Allison et al., 2005; Matamala et al., 2008). Overall, the current experimental design, which combined archived frozen soil cores from CBG and the chronosequence technique at Fermilab, enabled an opportunity to determine how differing soil origin and site history might influence below-ground succession over the restoration period.

Water-stable Aggregate Abundance

Soil macroaggregate abundance (0-10 cm) increased at Fermilab ($p<0.001$), while it declined at CBG over the period of interest ($p<0.05$) (Figure 15). At Fermilab, soil macroaggregate abundance seems to be reverting to remnant prairie conditions; aggregate abundance increased from 18.75% ($\sigma = 5.96$) in the 9-year-old plot to 58.95% ($\sigma = 6.03$) in the 34-year-old plot, a difference of 40.2 percentage points. Given a linear line of best fit, it will take approximately 50 years for the restoration to attain remnant conditions (73.13%; $\sigma = 2.75$), which corroborates previous studies at this site (Jastrow 1990). At CBG, in contrast, average aggregate abundance decreased from 19.69% ($\sigma = 4.37$) at 9 years post-prairie creation to 13.51% ($\sigma = 3.51$) at 26 years post-prairie creation, a difference of 6.81 percentage points. Interestingly, although the data reported an initial increase in soil aggregation to 35.45% ($\sigma = 5.21$) at 15 years post-prairie creation, it was followed by a decrease over the next 11 years. Since measurements were absent for initial conditions (the first data point is 9 years post-prairie creation), it cannot be determined whether a net increase or decrease occurred. The data lacks the desired degree of resolution (typical of this field of research); nevertheless, the results question the sustainability of prairies constructed at artificially created sites with non-native prairie soil.

Several methodological issues must be mentioned. First, soil samples from CBG were frozen to preserve them over the years, while those from Fermilab were refrigerated, since they were collected fresh from the field on a single date. The freeze/thaw process might have adversely affected particulate composition; however, at CBG, soil cores corresponding to 26 years post-prairie creation were freshly extracted and reported the lowest percent carbon values, so evidence of selective degradation was not evident. Second, dissimilar sampling dates were utilized; soil cores were collected during summer at Fermilab verses early winter at CBG, so the binding agents that glue macroaggregates together might be relatively degraded. If this were the case, a portion of aggregates would be unaccounted for within a smaller particle size fraction such as 250-425 μm, but the effect of this shortcoming is speculative.

Water-stable Aggregate Carbon Storage

Soil macroaggregate carbon storage (0-10 cm) was hypothesized to increase with restoration age at both sites, but this was not supported at CBG. Specifically, soil aggregate carbon storage increased at Fermilab ($p<0.001$) but decreased at CBG ($p<0.01$) (Figure 16). A significant interactive effect was reported in respect to site ($p<0.001$), which means that the coefficients of the best-fit lines were significantly different, lending support to different ecological trajectories. At Fermilab, macroaggregate carbon per unit soil increased from 0.99% ($\sigma = 0.02$) in the 9-year-old plot to 3.04% ($\sigma = 0.21$) in the 34-year-old plot, a difference of 2.05 percentage points, whereas the remnant plot reporting a value of 4.70% ($\sigma = 0.21$). Assuming a linear line of best fit, it will take approximately 60 years for the restoration to attain remnant conditions.

Given the limitations of the chronosequence technique, data trends are difficult to extrapolate (Fermilab occupies 452 ha); nevertheless, previous studies at Fermilab support these findings (Jastrow, 1990; Matamala et al., 2008). At CBG, the data reported a slight decrease in macroaggregate carbon storage from 1.46% ($\sigma = 0.29$) at 9 years post-prairie creation to 1.02% ($\sigma = 0.16$) at 26 years post-prairie creation. Macroaggregate carbon storage reached 2.82% ($\sigma = 0.40$) at 15 years post-prairie creation, but over the span of the next 11 years it dropped to 1.02%, the lowest value recorded for this site. Overall, site history and state factors are likely to modulate SOM integration into water-insoluble soil fractions. At CBG, the trajectory (or lack thereof) might be attributed to the non-native origin of the soil and/or the disturbances incurred to the soil during construction. Without a baseline value for CBG, however, it cannot be determined with certainty whether a net increase or decrease occurred over the restoration period.

Soil aggregate carbon concentration (% carbon by weight) increased at each site but was not significant at the $p<0.05$ level (Figure 16). The coefficients of the best-fit lines were similar, with values at CBG approximately 1.96% greater in carbon content. At Fermilab, macroaggregate carbon increased from 5.18% ($\sigma = 5.18$) in the agricultural field to 6.43% ($\sigma = 0.47$) in the 32-year-old plot, while the remnant plot reported a value of 6.42% ($\sigma = 1.46$). At CBG, the data reported an increase in carbon concentration from 6.83% ($\sigma = 1.46$) at 9 years post-restoration to 7.51% ($\sigma = 1.18$) in the 26 years post-restoration.

Since Fermilab was continuously cultivated for approximately 150 years, prolonged disturbance could have rendered soil aggregates relatively more degraded prior to restoration activities, a condition favorable to accrual over time. Also, inorganic particle composition should be considered: a previous study of two tallgrass prairie chronosequences in Wisconsin concluded

that fine textured soil has a greater propensity to store bulk soil carbon (Brye & Kucharik, 2003). Even though fine inorganic particles form chemical bonds with organic matter (Paul, 2007), differences would be impossible to explain, since both soils were classified as clay loam. Importantly, uncertainty exists if the methodology to measure inorganic particle composition had the resolution to adequately classify soil type, as standards were not calculated.

Previous research at Fermilab found a correlation between macroaggregate carbon concentration and diameter (Jastrow et al., 1996), but those results were not corroborated in this study. Relative to CBG, the diameter of macroaggregates at Fermilab was noticeably larger, yet the concentration was lower at any given point. Uncertainty exists as to whether sample storage influenced the results; at Fermilab, samples were stored in a laboratory refrigerator for approximately 2 months prior to aggregate isolation, which may have resulted in considerable microbial oxidation.

Soil macroaggregate C:N concentration was not statistically significant at either site, although the data would have little importance without values pertaining to the smaller, well-studied micoraggregate size classes. If smaller particle size classes were measured, then aggregate C:N could be a proxy for organic matter composition; for example, if carbon were low relative to nitrogen, this would suggest that aggregate binding agents such as root and microbial exudates (high C:N) are being preferentially oxidized, and/or higher quality inputs (low C:N) are being incorporated into aggregates.

WSA Carbon Storage and Associated Factors

A) Fungal abundance

AMF root colonization was hypothesized to increase with restoration age. Whereas AMF root colonization exhibited a statistically significant increase at Fermilab ($p<0.01$), no discernable trend existed at CBG (Figure 19). At Fermilab, the data reported an increase in AMF colonization from 75.62% ($\sigma = 4.59$) in the 9-year-old plot to 94.80% ($\sigma = 0.66$) in the 34-year-old plot. Interestingly, the lowest value was recorded in the remnant plot (70.89%; $\sigma = 3.30$), which is counterintuitive but might be an artifact of herbicide application that occurred several times over the last decade (R. Walton, pers. comm.). At CBG, AMF root colonization decreased from 77.82% ($\sigma = 1.47$) at 9 years post-prairie creation to 50.25% ($\sigma = 15.52$) at 26 years post-prairie creation. Worth mentioning is that the highest value was attained at 15 years post-restoration at 82.42% (σ = N/A), comparable to Fermilab's 19-year-old plot, with a value of 81.46% ($\sigma = 7.91$). Such a drastic change might be indicative of low statistical power, but, if the data were an accurate representation, it would reinforce AMF hyphae as a necessary condition for water-stable aggregate formation and preservation (Rillig, 2006).

For CBG, the net decrease in AMF colonization was interesting because previous observations documented a gradual loss in the abundance and diversity of C4 graminoids (D. Sollenberger, pers. comm.). Plant species such as *Andropogon gerardii* (bigbluestem, Poaceae) and *Sorghastrum nutans* (Indian grass, Poaceae), which require AMF mutualisms, decreased in abundance over the last decade, while *Spartina pectinata* (prairie cord grass, Poaceae), which is able to thrive in AMF suppressed soils, has spread throughout select regions of the prairie. Although quantitative data does not exist to trace above-ground species succession, it is plausible that the decline in warm season grasses is an above-ground response to acitivity within the soil. At Fermilab, a previous study found evidence that plant-neighbor interactions modulate root

morphology and AMF colonization (Jastrow & Miller, 1993), so aboveground plant species composition might have experienced this behavior.

Overall, the fungal data suffers from low sample size that makes it difficult to extrapolate the results across the macroscale, an experimental limitation that is nearly impossible to surmount given large land areas, but, nevertheless, the study's findings make sense when considering site history. In addition, factors that could not be controlled include the age effect (e.g., difference in colonization is a function of the age of the root stock), affinity to establish AMF mutualisms, and accurate representation of plant species composition. Lastly, the distribution of soil microbes is heterogeneous, so it is worth noting that data is highly influenced by a few select points where the soil cores were extracted.

B) Root Biomass and Substrate

Bulk root biomass was expected to increase with restoration age but, unfortunately, the hypothesis could not be properly tested. Soil core root harvesting technique is fundamentally flawed such that low sample size coupled with a narrow sampling diameter is not capable of capturing an accurate signal across the landscape. However, a previous study at Fermilab estimated plant biomass to be the first carbon stock to equilibriate after several decades of restoration management, so it is not likely to be a rate-limiting factor (Matamala et al., 2008). At CBG, when considering its shallow restrictive layer, which on average is 9 inches deep, as well as the presence of a perched water table, root development might be hindered in a vertical manner (D. Sollenberger, pers. comm.). Although CBG reported a steady decrease in root biomass from 0.86% ($\sigma = 0.55$) at 9 years post-prairie creation to 0.16% ($\sigma = 0.07$) at 26 years post-prairie creation, limited emphasis should be attributed to these results.

Fine root C:N was hypothesized to decrease with restoration age. Although a net decrease occurred at each site, the data was not significant at the $p<0.05$ level (Figure 20). At Fermilab, the data reported a decrease in fine root C:N from 66.28 (σ = N/A) in the 9-year-old plot to 34.60 (σ = N/A) in the 34-year-old plot, while the lowest value was recorded in the remnant site at 32.42 (σ = N/A). Similarly, CBG also reported a decrease in fine root C:N from 48.36 (σ = N/A) at 9 years post-prairie creation to 35.01 (σ = N/A) at 26 years post-prairie creation. At Fermilab, if the data captures the general trend, then an increase in root substrate palatability occurred over time, presumably due to greater fine root matter production, with a favorable affect on hetereotrophic foodwebs. A previous study at Fermilab reported root C:N ratio (<5 mm in diameter) to be lower at the remnant site (~70) when compared to the restored prairie plots (~90) (Matamala et al., 2008), which the authors attribute to age and the dominance of warm season grasses. At CBG, the results do not as a decrease in grass diversity abundance occurred; nonetheless, if root substrate quality has become more palatable at both sites, then carbon accrual should be favored. In the context of soil carbon storage, however, important to note is that root substrate quality is likely to represent a sufficient as opposed to a necessary condition. Worth noting, a significant difference was not supported between C3 and C4 fine root controls, both within and across sites. If a difference does exist, however, confounding variables such as the age effect might have masked a signal. Even so, root substrate quality is not sufficient because the amount of substrate deposited into the soil represents an important offset. Lastly, potential confounding factors must be identified, which include the age effect, root morphology, and root depth. Since the species of rootstock could not be identified, it is unknown if the sample was representative of the larger plant community. Also, it is uncertain if impurities were completely

removed during sample preparation (e.g., organic matter may have been encrusted on the surface of roots).

CBG was established on manmade islands along the Skokie Lagoons, so soil erosion might be an issue (D. Sollenberger, pers. comm.). On-site climate records, which date back to 1982, report several significant flooding events since 1997. Montly precipitation exceeded 10 cm during the following dates: September 1996 (11.3), August 1997 (19.2 cm), August 2000 (19.0 cm), October 2001 (11.2 cm), August 2007 (11.3 cm), and September 2008 (19.4 cm). Notably, in September 2008, which was the largest amount of monthly precipitation on record, the mesic section was submerged for approximately two days, so a considerable amount of SOM might have washed into the lagoons. If erosion is a valid concern, then clay concentration should increase with age, but this was not supported in the data.

Considering that the sites are located within 80 km of one another, temperature and precipitation patterns might have dissimilar effects on soil carbon cycling. Unfortunately, temperature records were difficult to interpret because average temperature was reported at Ferminlab verses average high and low temperature at CBG. Precipitation data was comparable, on the other hand, and a similar trend, with yearly precipitation at Fermilab reporting a less than 0.06 cm average difference on a monthly basis. To a degree, precipitation may be ruled out as have playing a significant role. However, rainwater percolation through the soil horizon is perhaps more important since CBG suffers from poor drainage, which, in turn, would have the ability to affect aeration and root development (D. Sollenberger, pers. comm.). Previous research at Fermilab found precipitation to modulate root development and that the soil is relatively well drained (Miller et al., 1995).

C) Bulk density and Electrical Conductivity

Soil bulk density (0-10 cm) was hypothesized to alleviate with restoration age. The results suggest compaction at Fermilab but alleviation at CBG. Specifically, soil bulk density exhibited a net increase at Fermilab ($p<0.001$) compared to a net decrease at CBG ($p<0.001$) (Figure 22). In addition, a significant interactive effect was reported in respect to site ($p<0.001$), so dissimilar ecological trajectories are supported. At Fermilab, bulk density decreased from 0.85 g/cm^3 ($\sigma = 0.002$) in the 9-year-old plot to 0.81 g/cm^3 ($\sigma= 0.001$) in the 34-year-old plot, with a remnant value of 0.82 g/cm^3 ($\sigma= 0.002$). At this point, it seems as though remnant conditions have already been achieved. In contrast, at CBG, carbon storage increased from 0.71 g/cm^3 ($\sigma = 0.013$) 9 years post-prairie creation to 0.75 g/cm^3 ($\sigma = 0.002$) 26 years post- prairie creation, a trend opposite to that of Fermilab.

Soil bulk density is a reflection of soil aggregation, AMF colonization, and root morphology, with a direct positive correlation between these factors and porosity (Paul, 2007). When considering soil macroaggregate abundance, the results are counterintuitive. For CBG, the magnitude doesn't make sense when considering the nature of disturbances incurred during construction. CBG has been consistently less dense but it has stored significantly less macroaggregate carbon. Perhaps the behavior resulted from sample collection in winter as well as freeze/thaw between sample storage and analysis. However, soil samples for 2008 were freshly extracted and conformed to the general trend, which does not support either possibility.

Even if the topsoil at CBG (0-10 cm) were less dense relative to Fermilab, it is important to consider hydrological activity while descending into the soil profile. Whereas the soil horizon at Fermilab is deep and relatively well drained, the soil at CBG has an average restrictive layer of 9 inches, which is supported by the presence of a perched water table during periods of high

precipitation (D. Sollenberger, pers. comm.). Over time, it is possible that hydrological factors and soil aggregation have facilitated a shift away from warm season grasses. At Fermilab, for example, *Andropogon Sorghastrum* dominate mesic soil *Spartina* thrive in wetter soils, while certain species such as *Gentiana puberulenta* (downy gentian, Gentianaceae) require water-stable aggregates for their establishment (Betz, 1996). Overall, feedback between soil moisture and below-ground processes may play an important role in the succession at each site.

Bulk soil electrical conductivity (EC) exhibited a statistically significant decrease at CBG ($p<0.001$), but no discernable trend was found at Fermilab (Figure 24). A significant interactive effect was reported in respect to site ($p<0.001$), with dissimilar ecological trajectories supported. At Fermilab, the data reported an electrical conductivity of 69.00 µS ($\sigma = 14.00$) in the agricultural plot, 57.00 µS ($\sigma = 32.08$) in the 34-year-old restoration plot, and 74.33 µS ($\sigma = 7.64$) in the remnant site. At CBG, the data reported a sharp decrease in electrical conductivity from 168.89 µS ($\sigma = 19.74$) at 9 years post-restoration to 74.67 µS ($\sigma = 5.68$) at 26 years post-restoration. Higher salinity at CBG might be attributed to its proximity to Interstate-94, but the sharp decrease in salinity is not easily understood. Overall, it is uncertain if pH and EC data can be extrapolated across the macroscale and, if so, whether it could significantly influence below-ground processes that interact with soil aggregation. Little emphasis can be placed without micronutrient values for Ca+, K+ and Na+.

Overall Site Functioning

The current study found evidence that Fermilab, as opposed to CBG, conformed to the general hypothesis of soil carbon storage. Specifically, Fermilab, increased in aggregate formation as well as soil carbon. At CBG, no discernable trend was evident, and number of

factors might have contributed to the peculiar activity such as the non-native origin of the soil, the disturbances incurred during construction, and hydrological issues. On a mechanistic level, multiple factors are likely to interact over spatial and temporal scales, which include a decrease in NPP, an increase in microbial oxidation, organic matter being shunted into soil fractions other than macroaggregates, or erosion of organic matter into the adjacent lagoons. Still, since the Fermilab restoration is 8 years older, perhaps considerable progress will occur at CBG within this timeframe. At Fermilab, during the first 25 years of restoration, the site recorded relatively low values for aggregate abundance, aggregate carbon per unit soil, and AMF colonization, with a considerable increase between 25 to 34 years post-restoration. Over the next decade or so, whether or not CBG improves its ability to store macroaggregate carbon is unclear.

Prairie Restorations and the Future

This study corroborates several concerns associated with soil carbon storage in tallgrass prairies. Previous research suggests that soil carbon sequestration rates are too low to offset atmospheric CO_2 on a respectable timescale (Schlesinger, 1990; Kucharik, 2007). In addition, the "carbon cost" incurred by management activities represents an important offset to carbon sink activity; over the restoration period, significant GHG emissions may be associated with transportation, irrigation, and artificial fertilizer application (Schlesinger, 2000). Since CBG is relatively small (6 ha) and undergoes intensive management, this suggests that soil carbon storage might be offset by its "carbon cost." Also, once a system has reached its carrying capacity, no further gains are made even though restoration management must continue.

In some instances, tallgrass prairie restorations may offset a considerable quantity of GHG emissions associated with fossil fuel combustion and land-use change. At artificially

created landscapes, however, land managers and key decision makers must recognize that site history, soil origin, and hydrological issues might inhibit sustainable carbon storage over time. Nevertheless, prairie restorations represent a valuable addition to a municipalities' "green" agenda when administered in tandem with recycling programs, stringent building codes, and energy saving initiatives, as restorations have the ability to create wildlife corridors and foster environmental awareness among residents that would otherwise not be exposed to native habitat.

A multi-faceted approach must be implemented to encourage sustainable soil carbon storage in the Midwest. When a prairie restoration or creation is not feasible, sustainable agriculture represents an alternate method to preserve and/or accrue soil carbon (Kucharik et al., 2007). Organic agriculture mitigates SOM depletion within soil aggregates by slowing their turnover rate (Baere, et al., 1994; Six et al., 2000; Kucharik et al., 2001), and these practices may incur a lower carbon cost when microbe-produced nutrients are used *in lieu* of synthetic fertilizer (Nardi, 2007).

Concluding Remarks

As a sustainable carbon reservoir, the macroaggregate soil fraction promotes healthy above- and below-ground functioning and, in some cases, offsets GHG emissions. The results of this study suggest that prairies constructed on artificially created sites might not be effective at storing macroaggregate carbon over several decades of management. Although low statistical power and confounding variables prevent robust conclusions, the findings are compelling and should spur future lines of research. In a context greater than soil carbon storage, land managers should be attentive to site history and soil origin in order to implement best practice restoration techniques.

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APPENDIX A

Site	Age (years)	% Sand	% Clay	% Silt
CBG	9	44.4	48.6	7
CBG	9	38.8	50	11.2
CBG	9	38	52	10
CBG	15	54.4	43	2.6
CBG	15	46.4	48	5.6
CBG	15	44.4	50	5.6
CBG	18	42.4	50	7.6
CBG	18	48.4	48	3.6
CBG	18	42.4	50.4	7.2
CBG	26	42.4	49	8.6
CBG	26	42.4	49	8.6
CBG	26	42.4	49	8.6
FL	AG	48.4	44.4	7.2
FL	AG	41.2	46	12.8
FL	AG	45.2	46	8.8
FL	9	39.2	50	10.8
FL	9	37.2	54	8.8
FL	9	37.2	40.4	22.4
FL	19	38.4	52.8	8.8
FL	19	36.4	48.8	14.8
FL	19	36.4	52	11.6
FL	21	29.2	54	16.8
FL	21	31.2	54	14.8
FL	21	34.4	56	9.6
FL	25	40.4	50.4	9.2
FL	25	37.4	52.4	10.2
FL	25	34.4	52	13.6
FL	28	44.6	46.2	9.2
FL	28	37.4	48.4	14.2
FL	28	37.4	46.4	16.2
FL	32	54.6	42.8	2.6
FL	32	45.6	46.8	7.6
FL	32	37.6	46.8	15.6
FL	34	38.8	47.2	14
FL	34	39.6	48.2	12.2
FL	34	45.6	48.8	5.6
FL	R	43.2	45	11.8
FL	R	48.4	44.8	6.8
FL	R	43.2	45.6	11.2

Table 43. Raw data for inorganic particle compostion.

Site	Age (years)	Aggregates/unit soil (%)
CBG	9	24.61
CBG	9	25.39
CBG	9	19.33
CBG	9	17.20
CBG	9	14.66
CBG	9	16.97
CBG	15	29.90
CBG	15	30.23
CBG	15	36.23
CBG	15	36.19
CBG	15	44.18
CBG	15	35.97
CBG	18	20.55
CBG	18	14.56
CBG	18	23.61
CBG	18	20.70
CBG	18	16.09
CBG	18	21.08
CBG	26	18.20
CBG	26	12.16
CBG	26	10.23
CBG	26	17.61
CBG	26	12.26
CBG	26	10.59
FL	AG	34.68
FL	AG	27.28
FL	AG	25.48
FL	9	16.31
FL	9	25.55
FL	9	14.40
FL	19	14.61
FL	19	19.48
FL	19	25.54
FL	21	31.71
FL	21	24.40
FL	21	15.71
FL	25	24.95
FL	25	25.60
FL	25	37.53
FL	28	48.55
FL	28	56.40
FL	28	52.36
FL	32	51.13
FL	32	75.33
FL	32	53.98

FL	34	59.35
FL	34	64.77
FL	34	52.72
FL	R	76.06
FL	R	70.61
FL	R	72.72

Table 44. Raw data for soil water-stable aggregate abundance (%).

Site	Age (years)	Total C %	Total N %	C/N ratio	% agg (g)/ g soil
CBG	9	6.86	0.37	18.62	1.35
CBG	9	8.35	0.57	14.53	1.65
CBG	9	8.07	0.53	15.08	1.59
CBG	9	8.40	0.52	16.13	1.66
CBG	9	5.55	0.25	22.63	1.09
CBG	9	5.42	0.09	58.41	1.07
CBG	9	5.12	0.09	58.75	1.82
CBG	15	8.41	0.57	14.68	2.98
CBG	15	8.43	0.54	15.60	2.99
CBG	15	9.08	0.57	15.98	3.22
CBG	15	6.04	0.31	19.70	2.14
CBG	15	6.66	0.33	20.07	2.36
CBG	15	8.82	0.60	14.82	3.13
CBG	15	8.14	0.50	16.39	2.89
CBG	18	8.42	0.63	13.29	1.64
CBG	18	4.31	0.14	31.24	0.84
CBG	18	4.67	0.11	42.18	0.91
CBG	18	6.96	0.35	19.85	1.35
CBG	18	6.22	0.23	26.88	1.21
CBG	18	7.05	0.37	19.14	1.37
CBG	18	9.01	0.60	15.13	1.75
CBG	26	7.63	0.42	18.31	1.03
CBG	26	7.14	0.40	17.92	0.96
CBG	26	7.42	0.41	18.14	1.00
CBG	26	5.59	0.25	22.70	0.76
CBG	26	7.70	0.39	19.76	1.04
CBG	26	9.64	0.65	14.83	1.30
CBG	26	7.49	0.39	19.11	1.01
FL	AG	5.15	0.45	11.35	1.50
FL	AG	5.04	0.45	11.08	1.47
FL	AG	5.34	0.44	12.24	1.56
FL	9	5.30	0.38	13.99	0.99
FL	9	5.39	0.39	13.92	1.01
FL	9	5.14	0.38	13.51	0.96
FL	19	4.80	0.36	13.16	0.95
FL	19	4.69	0.34	13.65	0.93

FL	19	5.14	0.40	12.72	1.02
FL	21	3.23	0.24	13.27	0.77
FL	21	3.93	0.27	14.60	0.94
FL	21	4.32	0.30	14.36	1.03
FL	25	5.29	0.40	13.17	1.55
FL	25	5.82	0.44	13.29	1.71
FL	25	5.29	0.41	13.03	1.55
FL	28	5.93	0.46	12.84	3.11
FL	28	6.13	0.47	13.14	3.21
FL	28	5.24	0.41	12.66	2.75
FL	32	6.40	0.49	12.99	3.85
FL	32	5.97	0.47	12.65	3.59
FL	32	6.91	0.53	13.00	4.15
FL	34	5.40	0.39	13.73	3.18
FL	34	4.75	0.36	13.10	2.80
FL	34	5.32	0.39	13.59	3.13
FL	R	6.33	0.52	12.21	4.63
FL	R	6.47	0.42	15.49	4.74
FL	R	6.45	0.46	13.90	4.72

Table 45. Raw data for water-stable aggregate elemental composition data.

Site	Age (years)	Total C %	Total N %	Avg. C:N ratio
CBG	9	44.81	0.93	48.36
CBG	15	39.45	1.17	33.61
CBG	18	40.15	1.36	29.53
CBG	26	42.13	1.20	35.01
FL	9	43.22	0.65	66.28
FL	19	37.41	1.07	35.02
FL	21	39.6	0.61	64.54
FL	25	37.81	1.04	36.48
FL	28	38.72	1.03	37.51
FL	32	41.29	0.84	49.40
FL	34	32.38	0.94	34.60
FL	R	41.83	1.29	32.42

Table 46. Raw data for fine root (<2mm diameter) elemental composition data.

Site	Species	Total C %	Total N %	C:N
CBG	Spartina	42.56	1.19	35.76
CBG	Spartina	42.8	1.02	41.96
CBG	Spartina	43.9	1.11	39.55
CBG	Solidago	40.25	1.50	26.83
CBG	Solidago	40.98	1.59	25.77
CBG	Solidago	43.38	1.05	41.31
FL	Sorghum	41.58	1.41	29.49
FL	Sorghum	41.87	1.65	25.38
FL	Sorghum	44.81	0.86	52.10
FL	Solidago	41.66	1.04	40.06
FL	Solidago	44.03	0.72	61.15
FL	Solidago	44.01	1.15	38.27

Table 47. Raw data for C3 and C4 fine root control elemental composition data.

Site	Age (years)	pH	EC
CBG	9	7.2	185
CBG	9	7.8	257
CBG	9	7.2	180
CBG	9	6.9	117
CBG	9	7.5	170
CBG	9	8	145
CBG	9	7	258
CBG	9	8.1	116
CBG	15	8	92
CBG	15	7.2	122
CBG	15	7.6	122
CBG	15	6.8	101
CBG	15	7.5	145
CBG	15	7.3	146
CBG	15	7.3	166
CBG	15	7.6	122
CBG	15	6.9	186
CBG	15	7.6	65
CBG	18	7.4	167
CBG	18	7.8	91
CBG	18	9.1	92
CBG	18	8.3	101
CBG	18	7.6	110
CBG	18	7.6	119
CBG	18	7	229
CBG	18	7.4	196
CBG	18	7.8	152
CBG	18	7.5	203
CBG	26	7.8	88
CBG	26	7.7	63

CBG	26	7.9	56
CBG	26	7.8	54
CBG	26	7.6	95
CBG	26	7.7	60
CBG	26	7.4	85
CBG	26	7.3	97
CBG	26	7.4	74
FL	AG	7.4	63
FL	AG	7.5	85
FL	AG	7.5	59
FL	9	7.6	31
FL	9	7.7	34
FL	9	7.7	30
FL	19	7.7	37
FL	19	7.2	29
FL	19	7.1	52
FL	21	7	27
FL	21	6.8	29
FL	21	6.9	24
FL	25	7.5	38
FL	25	7.1	65
FL	25	6.9	36
FL	28	7.8	55
FL	28	7.7	50
FL	28	7.5	75
FL	32	6.9	51
FL	32	7	56
FL	32	6.8	48
FL	34	8	94
FL	34	7.4	40
FL	34	7.3	37
FL	R	7.5	66
FL	R	7.2	76
FL	R	7.2	81

Table 48. Raw data for bulk soil pH and Electrical Conductivity (EC) measurements.

Site	Age (years)	Bulk density (g/cm^3)
CBG	9	0.71
CBG	9	0.71
CBG	9	0.68
CBG	9	0.73
CBG	9	0.71
CBG	9	0.71
CBG	9	0.71
CBG	15	0.74
CBG	15	0.73
CBG	15	0.74
CBG	15	0.74
CBG	15	0.74
CBG	15	0.75
CBG	18	0.76
CBG	18	0.76
CBG	18	0.75
CBG	18	0.75
CBG	18	0.75
CBG	18	0.75
CBG	26	0.76
CBG	26	0.76
CBG	26	0.75
CBG	26	0.75
CBG	26	0.75
CBG	26	0.76
FL	AG	0.84
FL	AG	0.83
FL	AG	0.84
FL	9	0.85
FL	9	0.85
FL	9	0.85
FL	19	0.84
FL	19	0.84
FL	19	0.85
FL	21	0.85
FL	21	0.85
FL	21	0.85
FL	25	0.82
FL	25	0.83
FL	25	0.83
FL	28	0.82
FL	28	0.82
FL	28	0.82
FL	32	0.80
FL	32	0.80

FL	32	0.80
FL	34	0.81
FL	34	0.81
FL	34	0.81
FL	R	0.82
FL	R	0.81
FL	R	0.82

Table 49. Raw data for soil bulk density measurements.

Site	Age (years)	% Root biomass/unit soil	Site	Age (years)	% Root biomass/unit soil
FL	AG	0.07	CBG	9	1.36
FL	AG	0.00	CBG	9	0.32
FL	AG	0.16	CBG	9	0.14
FL	AG	0.13	CBG	9	1.37
FL	AG	0.08	CBG	9	0.71
FL	9	2.48	CBG	9	1.27
FL	9	6.30	CBG	15	0.27
FL	9	3.57	CBG	15	0.06
FL	9	13.31	CBG	15	0.53
FL	9	8.82	CBG	15	0.97
FL	19	6.76	CBG	15	0.14
FL	19	8.19	CBG	15	0.34
FL	19	9.13	CBG	18	0.48
FL	19	10.08	CBG	18	0.75
FL	21	1.11	CBG	18	0.53
FL	21	1.96	CBG	18	0.06
FL	21	2.46	CBG	18	0.48
FL	21	0.80	CBG	18	0.39
FL	21	2.26	CBG	26	0.26
FL	25	7.53	CBG	26	0.06
FL	25	5.29	CBG	26	0.12
FL	25	6.98	CBG	26	0.22
FL	25	6.81	CBG	26	0.15
FL	25	11.65	CBG	26	0.15
FL	28	6.62			
FL	28	6.15			
FL	28	6.27			
FL	28	3.53			
FL	32	1.06			
FL	32	1.07			
FL	32	2.64			
FL	32	2.62			
FL	32	1.96			
FL	34	9.53			

FL	34	17.04			
FL	34	11.36			
FL	34	6.64			
FL	34	6.28			
FL	R	1.47			
FL	R	1.73			
FL	R	0.83			
FL	R	0.66			
FL	R	0.51			

Table 50. Raw data for bulk root biomass (%) measurements.

Site	Year	Total precip (cm)	Apr-Oct Precip (cm)	Avg. high temp (°C)	Apr-Oct avg. high temp (°C)	Avg. low temp (°C)	Apr-Oct avg. low temp (°C)	Days above 90 °F	Largest rainfall (cm)
CBG	1991	90.45	65.30	15.22	23.22	5.33	12.32	28	7.77 Oct 4-5
CBG	1992	81.05	48.16	13.22	19.89	4.28	9.46	3	5.92 Nov 1-2
CBG	1993	96.34	75.59	13.39	20.93	4.28	10.76	7	5.16 Jun 8-9
CBG	1994	84.25	46.56	14.72	22.56	4.22	11.22	21	11.61 Jun 24-25
CBG	1995	99.90	66.17	14.17	22.42	4.44	11.81	17	7.80 Apr 25-30
CBG	1996	90.58	73.18	13.28	21.09	3.06	9.96	12	22.12 May 6-29
CBG	1997	95.83	58.88	13.89	21.22	3.94	9.98	11	6.65 Aug 15-18
CBG	1998	89.56	61.60	16.44	23.23	6.56	12.27	19	7.95
CBG	1999	92.66	69.37	15.83	22.89	4.67	12.21	16	5.51 Apr 23
CBG	2000	110.39	70.33	15.06	22.41	4.83	11.67	8	7.49 Apr 19-21
CBG	2001	112.52	92.48	15.72	22.80	5.00	11.30	19	7.95 Oct 12
CBG	2002	85.47	65.58	15.61	22.79	4.89	11.11	30	13.08 Aug 21-22
CBG	2003	80.59	55.75	14.56	21.83	3.50	9.95	15	5.66 May 1
CBG	2004	90.30	61.34	15.11	22.44	4.44	10.58	5	11.02 May 21-22
CBG	2005	62.05	32.39	15.61	23.78	5.28	12.17	24	2.74 Jul 4
CBG	2006	108.13	72.82	15.72	22.28	5.44	11.18	15	9.12 Oct 2
CBG	2007	104.14	70.97	15.44	23.67	4.72	11.68	18	6.17 Aug 27
CBG	2008	125.88	80.52	14.28	22.38	3.72	11.17	6	8.51 Sep 14
	Average	94.46	64.82	14.85	22.32	4.59	11.16	15.22	

Table 51. Descriptive statistics for yearly temperature and precipitation data at CBG (1991-2008).

Site	Year	Total precip (cm)	Apr-Oct precip (cm)	Avg. temp (°C)	Apr-Oct temp (°C)
Fermilab	1995	91.21	69.47	8.93	16.85
Fermilab	1996	89.15	74.17	7.87	15.57
Fermilab	1997	71.37	48.77	8.42	15.59
Fermilab	1998	86.11	65.79	11.11	17.58
Fermilab	1999	83.82	63.75	10.02	17.06
Fermilab	2000	80.26	63.50	9.38	16.73
Fermilab	2001	69.34	53.06	9.73	16.55
Fermilab	2002	99.64	86.39	9.79	16.76
Fermilab	2003	75.74	53.92	8.97	16.11
Fermilab	2004	74.70	50.11	9.50	16.44
Fermilab	2005	51.59	29.39	10.11	18.01
Fermilab	2006	97.00	70.13	10.32	16.68
Fermilab	2007	82.83	59.56	9.92	17.79
Fermilab	2008	106.25	83.92	8.54	16.41
	Average	82.78	62.28	9.47	16.72

Table 52. Descriptive statistics for yearly temperature and precipitation data at Fermilab (1995-2008).

Site	Year	April	May	June	July	August	Sept	October	Average
Fermilab	1994	12.22	14.61	20.89	21.39	19.28	17.50	9.17	16.44
Fermilab	1995	7.11	14.22	21.44	23.72	24.33	15.72	11.39	16.85
Fermilab	1996	7.00	12.94	20.39	20.44	21.50	16.22	10.50	15.57
Fermilab	1997	6.89	11.94	20.44	22.00	19.94	16.94	10.94	15.59
Fermilab	1998	9.44	18.17	20.17	22.28	22.11	19.28	11.61	17.58
Fermilab	1999	9.89	16.61	20.94	25.00	20.22	16.28	10.50	17.06
Fermilab	2000	8.72	16.67	19.44	21.11	21.61	17.06	12.50	16.73
Fermilab	2001	11.56	15.72	19.44	21.11	21.61	15.83	10.56	16.55
Fermilab	2002	9.67	12.72	21.17	24.11	22.06	18.61	9.00	16.76
Fermilab	2003	9.06	13.61	18.72	21.94	22.22	16.61	10.56	16.11
Fermilab	2004	10.28	15.83	19.56	21.06	18.78	18.17	11.39	16.44
Fermilab	2005	11.06	13.78	23.22	23.44	22.61	19.89	12.06	18.01
Fermilab	2006	11.72	15.00	19.67	23.78	21.94	16.00	8.67	16.68
Fermilab	2007	7.89	17.61	21.39	21.83	22.61	18.94	14.28	17.79
Fermilab	2008	9.39	13.44	21.00	22.39	20.83	17.56	10.22	16.41
	Average	9.46	14.86	20.53	22.37	21.44	17.37	10.89	

Table 53. Raw data for average temperature (°C) at Fermilab during the growing season.

Site	Year	April	May	June	July	August	Sept	October	Average
Fermilab	1994	3.30	2.03	14.48	3.81	18.54	4.06	5.59	7.39
Fermilab	1995	14.43	11.61	5.66	8.66	13.36	4.32	11.43	9.93
Fermilab	1996	5.33	15.24	12.45	26.92	3.56	4.57	6.10	10.59
Fermilab	1997	5.59	5.84	12.19	2.03	11.18	5.08	6.86	6.96
Fermilab	1998	11.18	8.89	8.13	10.41	8.38	4.83	13.97	9.40
Fermilab	1999	16.51	11.43	13.46	9.91	3.56	6.60	2.29	9.12
Fermilab	2000	12.70	9.14	11.94	9.40	6.35	9.65	4.32	9.07
Fermilab	2001	3.81	7.37	6.48	3.12	6.10	12.32	13.87	7.57
Fermilab	2002	10.08	13.89	15.85	26.09	13.59	2.54	4.34	12.34
Fermilab	2003	10.13	13.87	4.14	12.32	3.71	5.36	4.39	7.70
Fermilab	2004	3.48	15.16	9.58	4.90	8.38	0.79	7.82	7.16
Fermilab	2005	5.61	6.20	1.04	4.90	5.87	4.22	1.55	4.19
Fermilab	2006	9.55	11.07	10.01	4.32	7.39	12.80	14.99	10.01
Fermilab	2007	8.61	2.26	7.59	10.26	20.68	4.24	5.92	8.51
Fermilab	2008	6.99	10.52	10.77	9.40	5.66	31.12	5.23	11.38
	Average	8.48	9.63	9.58	9.75	9.09	7.49	7.24	

Table 54. Raw data for total precipitation (cm) at Fermilab during the growing season.

Site	Year	April	May	June	July	August	Septemb er	October	Average
CBG	1991	15.18	23.39	26.96	29.93	28.17	22.59	16.33	23.22
CBG	1992	11.17	19.28	22.78	24.52	24.23	21.63	15.66	19.89
CBG	1993	11.13	21.06	23.74	27.88	27.42	19.57	15.73	20.93
CBG	1994	15.82	20.34	26.63	28.51	25.68	24.00	16.97	22.57
CBG	1995	11.32	19.08	27.07	29.98	29.57	22.57	17.33	22.42
CBG	1996	11.74	16.67	24.96	26.67	28.01	22.85	16.81	21.10
CBG	1997	12.50	16.77	24.91	27.97	25.25	23.43	17.67	21.22
CBG	1998	13.82	22.78	25.65	28.03	27.99	26.48	17.79	23.22
CBG	1999	13.52	21.63	26.21	31.18	26.04	24.04	17.69	22.90
CBG	2000	13.33	21.61	24.82	26.31	27.69	23.89	19.19	22.41
CBG	2001	17.17	21.20	25.32	28.73	28.12	22.54	16.49	22.79
CBG	2002	14.61	18.04	27.35	30.25	28.42	25.82	14.98	22.78
CBG	2003	13.13	17.09	24.54	28.26	28.66	23.57	17.56	21.83
CBG	2004	15.78	21.34	25.02	26.77	25.16	25.68	17.37	22.44
CBG	2005	15.67	19.39	29.28	28.96	28.80	26.56	17.81	23.78
CBG	2006	16.76	20.98	24.68	29.41	27.99	21.83	14.29	22.28
CBG	2007	12.89	22.54	26.82	28.69	28.55	26.04	20.11	23.66
CBG	2008	14.57	19.08	26.78	28.49	27.42	23.89	16.42	22.38
	Average	13.89	20.13	25.75	28.37	27.40	23.72	17.01	

Table 55. Raw data for high average temperature (^{o}C) at CBG during the growing season.

Site	Year	April	May	June	July	August	September	October	Average
CBG	1991	10.03	10.13	3.15	2.69	8.89	9.45	20.96	9.32
CBG	1992	7.59	1.50	3.05	12.14	9.25	10.21	4.42	6.88
CBG	1993	13.67	8.10	20.29	8.48	12.57	9.63	2.84	10.80
CBG	1994	6.60	2.82	17.35	5.13	8.79	2.92	2.95	6.65
CBG	1995	16.84	11.05	1.04	10.31	11.00	4.93	11.00	9.45
CBG	1996	7.34	22.38	12.19	12.09	5.05	7.95	6.17	10.46
CBG	1997	4.39	10.74	9.53	10.11	12.83	5.13	6.15	8.41
CBG	1998	10.29	8.15	12.52	5.23	7.01	3.45	14.94	8.79
CBG	1999	22.68	6.60	12.22	4.88	10.97	8.99	3.02	9.91
CBG	2000	11.84	11.15	15.54	9.98	6.48	10.19	5.16	10.06
CBG	2001	7.16	12.14	10.01	6.76	22.28	13.39	20.75	13.21
CBG	2002	7.52	12.85	9.37	3.48	20.70	8.64	3.02	9.37
CBG	2003	6.45	19.89	3.05	13.06	3.02	5.13	5.16	7.98
CBG	2004	3.68	23.93	8.59	7.95	9.93	0.58	6.68	8.76
CBG	2005	2.72	4.55	1.98	7.70	5.89	7.19	2.36	4.62
CBG	2006	8.03	9.32	7.87	6.12	8.36	13.44	19.69	10.41
CBG	2007	10.54	5.56	5.87	6.68	32.41	5.00	4.90	10.13
CBG	2008	9.80	8.51	8.43	11.84	3.05	32.33	6.55	11.51
	Average	9.30	10.52	9.02	8.03	11.02	8.81	8.15	

Table 56. Raw data for total precipitation (cm) at CBG during the growing season.