THE PRAIRIE UNDERGROUND:
SOIL RECOVERY IN CHICAGO WILDERNESS RESTORATION

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

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"The soil is the great connector of lives, the source and destination of all. It is the healer and restorer and resurrector, by which disease passes into health, age into youth, death into life. Without proper care for it we can have no community, because without proper care for it we can have no life."

- Wendell Berry

Abstract

The tallgrass prairie once dominated much of the mid-western United States. Today, this highly productive system has been severely reduced, contributing to an array of environmental problems. The recent renaissance in natural area restoration and stewardship of the tallgrass prairie in the Chicago Wilderness region (Illinois, USA), especially in the efforts to restore former agricultural fields to tallgrass prairie, offer a valuable opportunity to assess the generality of changes in soil ecosystem-level processes in managed grassland systems. In this study, I examine the changes in the abundance of fungi relative to bacteria (F: B, by qPCR) and physical and chemical factors (texture, aggregate structure, acidity, soil organic matter (SOM), nitrogen (N) and phosphorous (P) fertility) in soils collected from prairie restorations of varying ages, as well as abandoned fields and undisturbed native prairie remnants. I used these data to ask whether shifts in microbial abundance and community structure occur within aggrading soil systems, and how these shifts might be related to other soil factors. More specifically, I predicted that the abundance of fungi relative to bacteria should increase following conversion from tillage agriculture to tallgrass prairie, and continue to increase with increases in SOM over the management period. If so, the detection of a substantial increase in microbial biomass and F: B could be used to indicate effective restoration progress, or reflect changes in other indicators of soil function. My results demonstrate that F: B were lower in abandoned fields than in restored sites, and that F:B was related to soil fertility and other edaphic properties. These data support my prediction, and indicate that management on a regional scale promotes a shift in microbial community structure. This finding is important because it suggests that fungi play an increasingly significant role in soil biological processes of managed and restored grasslands. Further, the observed shifts in F: B were correlated with decreasing soil fertility (N) and changes in other edaphic properties, such as silt and pH. The shift in F: B was not substantially correlated with soil aggregation, clay, or P. While these results support my predictions, they also suggest that changes in abiotic soil properties may be a prerequisite for increases in fungal biomass toward levels found in pristine prairie remnants. Overall, these findings lay an important foundation for understanding tallgrass prairie restoration and management in that: deficits in the microbial community structure resulting from intensive agriculture may be reversible; restoration management may facilitate the shift to a fungal-based soil food web; and F: B ratios provide a valuable integrative measure of system development and maturity.

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Introduction

The species-diverse grasslands of the North American prairie are renowned for their deep black soils with excellent structure, high organic content, and flourishing abundance of macro- and micro-flora and -fauna. However, much of the original prairie grassland has been lost to agriculture and commercial and residential development, and only a few remnant prairies now remain within a highly fragmented landscape. While efforts to restore former farmland to prairie have gained momentum, the measures of restoration success are usually based only on aboveground plant community metrics. Comparable markers of belowground restoration success and the congruence between the below- and aboveground markers in restoration are less well known. Thus, the overarching objective of this study was to examine the dynamics of belowground soil recovery in restored grasslands throughout the Chicago Wilderness region. Using a chronosequence approach, I investigated the impacts of prolonged land management on restored grassland soil properties, including soil physical (texture, aggregation, bulk density), chemical (C, N, PO_4, pH, moisture) and biological (microbial biomass, fungal: bacterial ratio) attributes to determine the belowground *precision* and *accuracy* of land managers' vegetation-based determination of restoration success along a management sequence.

Conceptual model of prairie soil recovery

My study incorporates a model of prairie soil recovery that is based on the relationships between soil quality components (Figure 1). In this model, soil quality is represented by groupings of certain physical, chemical and biological soil components typical of differing stages of prairie development and maturity. These groupings of soil quality components occur along a spectrum, ranging from very degraded sites, on the one extreme, to highly developed and mature

sites, on the other (Figure 2). This model suggests that degraded prairie soils may be diminished in aggregate structure and carbon storage, and have greater nutrient loads (particularly nitrogen and phosphate), perhaps due to the agricultural legacy of previous applications of synthetic fertilizer. In these conditions, aggressive and opportunistic plants may thrive. These degraded soil environments may also encourage the growth of bacteria that thrive on high nitrogen levels beneath the surface, and promote bacteria-based food webs. Highly developed and mature prairie, on the other hand, may harbor greater microbial biomass and fungal abundance in relation to bacteria. Such fungi would include both saprobes, which help decompose and recycle nutrients, and mutualists, which help plants to mine scarce water and nutrients. As the roots of mature-system prairie plants and the hyphae of their associated mutualistic fungi grow through the soil, they wrap around soil particles and build up aggregate structure, and in turn increase carbon storage and water infiltration (Figure 2). This conceptual model forms the basis of my investigation.

The tallgrass prairie: regional significance, ecosystem functioning, and the consequences of degradation

Tallgrass prairies ecosystems are a subgroup of temperate grasslands that once dominated the Great Plains of North America (Ceballos et al. 2010, Jones & Cushman 2004). Growing atop the glacial drift, glacial till, and windblown silt loess deposited in the wake of the Wisconsinan Glaciation, the deep roots of Midwest tallgrass prairie plants, as well as their associated microbial communities, built up a rich matrix of fertile black soils (Schwegman 1973). These prairie soils, termed Mollisols, serve important environmental needs, such as preventing erosion, facilitating hydrological processes, and acting as a "bank" to store carbon (Kucharik 2006,

2007). Beginning in the early 1800s, with the advent of new agricultural technology (Cochrane 1993), and continuing through the present, prairies have been, and are being, plowed and paved over to make way for the needs of the growing human population. Today, tallgrass prairie systems have been severely reduced, both in global abundance and within the North American Continent (Knopf and Samson 1994), by intensive agricultural practices and urban and suburban development (Iverson 1988). In Illinois, for example, it is estimated that only 0.1%, or approximately 2,300 acres, of high-quality pre-settlement tallgrass prairie remain (IDNR 2007, Anderson 1991). The loss of prairie to development and intensive agriculture has diminished the ability of these ecosystems to conduct environmental services for humanity (such as erosion protection, carbon storage, and water infiltration and retention), which may exacerbate several of the pressing environmental challenges facing human society today, such as soil loss and degradation (Pimentel et al. 1995), rising levels of atmospheric carbon dioxide (Smith et al. 2009), and the depletion and contamination of belowground moisture reserves (Pimentel et al 1995, Crosson et al. 1995). Additionally, many plants, insects, fungi and animals are endemic to these grassland habitats (Panzer et al. 1991, 1995, Risser 1988, Anderson 1991, Herkert 1991). Thus, prairies restoration is an important component of global strategies to conserve biodiversity, and might represent one way in which to stem the rapid declines in biodiversity that have accompanied the proliferation of human society (Pimm et al. 1995).

Restoration activity in the Chicago Wilderness

In the Chicago region (Illinois, USA), only a small portion of the original native tallgrass prairie lands, approximately 100 acres, remains today (Chicago Wilderness 1999). The conservation community has been working to purchase these original prairies for wildlife

conservation and to maintain the terrestrial plant communities as prairie (The Nature Conservancy 2014, Rowe et al. 2013). In 1996, a group of 34 private, non-profit, and governmental organizations, along with many caring volunteers, banded together in a regional alliance, called Chicago Wilderness, to protect, restore, and study the natural ecosystems in the greater Chicago metropolitan area (Chicago Wilderness 2014, Moskovits et al. 2002). Since then, there has been a renaissance in natural area restoration and stewardship around Chicago, so much so that the Chicago Wilderness alliance has grown to include over 300 different regional government, non-profit, and private organizations (Chicago Wilderness 2014). Recently, one focus of Chicago Wilderness was to establish and study a network of restored and remnant prairies, woodlands and savannas. This has been called the Chicago Wilderness Land Management Research Program (CWLMRP; Heneghan et al. 2012), commonly referred to as "100 Sites for 100 years". The respective owners of these natural areas agreed to maintain the lands and their current management treatment, in perpetuity. These Chicago-region efforts to restore and manage tallgrass prairie offer a valuable opportunity to assess the generality of changes in ecosystem-level processes in managed grassland systems. Equipped with such understanding of the influences of restoration and management on prairie system processes, land managers may be better suited to improve restoration outcomes.

The overall objective of scientists working within the CWLMRP initiative is to evaluate the impacts of management on aboveground and functional aspects of natural areas around the Chicago region, including pollinators, invertebrates, and plant community structure (Tonietto *in progress*, McCreary *in progress*, Martinez *in progress*, Umek *in progress*, Heneghan 2012,). However, two-thirds of a prairie system lies belowground (State of Minnesota 1998), thereby making the belowground system a critical component of prairie ecology (Heneghan et al. 2008,

Callaham et al. 2008) that needs to be both understood and addressed in restoration and management of these whole-grassland systems. While limited baseline soil properties within the CWLMRP network were previously established by CWLMRP scientists (Heneghan et al. 2012), there is little to no in-depth and synthesized information on the impact of prolonged management practices on soil quality in these restorations. Thus, there remains a large knowledge gap in our understanding of how closely the soil systems of even the most valued prairie restorations resemble those of original native prairies remnants. My study helps fill this knowledge gap by generating data that expands upon and gives higher resolution and statistical power to current CWLMRP prairie soil data, and is one of the first inquiries to synthesize and discuss this information in terms of target restoration outcomes.

Targets for restoration outcomes

At its onset, the CWLMRP project surveyed land managers in the Chicago region and asked them to identify pristine prairie remnants and reference prairie restorations that would represent high-quality targets for restoration outcomes[1]. These targets were primarily chosen based on similar, high-quality[2] aboveground characteristics[3]. However, it is unclear how precisely the belowground quality of soil mirrors the vegetation-based targets. In other words, while the aboveground characteristics of target prairies may be similar (in that they all meet qualifications for selection as a "target"), the belowground similarity of these target prairies has not been established. It is possible that two seemingly similar prairie targets, based on

[1] This work surveying regional land managers is still ongoing. Therefore, the current availability of individual site-history information is variable and patchy. (See Table 6 in the Appendix.)
[2] "High-quality", as used here, is a subjective term, based on each land manager's respective expertise.
[3] Further research into individual land managers' selection processes would further verify this assumption.

aboveground vegetation, are actually quite different, when belowground soil quality is incorporated into an evaluation of the prairie. That the criteria for target prairie selection did not incorporate belowground soil quality[4], and was rather based primarily on aboveground vegetative characteristics[5], raises questions about the belowground accuracy of vegetation-based target designations. This is the first study to examine and evaluate the link between aboveground site classifications and belowground soil properties to determine the precision and accuracy with which "target" prairies within the CWLMPR natural areas network are identified.

The importance of belowground aspects of whole-prairie recovery: soil quality indicators

Prairies are complex, interacting systems. Thus, it is crucial to consider both above- and belowground ecosystem characteristics, and feedbacks between the two, to gain a better understanding of these dynamic systems (Wardle 2004, Heneghan et al. 2008). The soil microbial community is a critical component of belowground ecology in prairie communities. The mechanisms by which microbial communities regulate organic matter decomposition and related nutrient cycling and availability are well studied (van Veen et al. 1984, Wardle 1992, Scow 1997, Lovell 1995). Additionally, that shifts within the microbial community structure can feed back to influence the composition of larger soil fauna, particularly fungal-feeding animals is widely accepted (Yeates 1997, Coleman and Whitman 2005). What is less well known, however, is the extent to which fungi and bacteria are differentially related to these processes, especially in systems undergoing restoration.)

[4] Further research into individual land managers' selection processes would further verify this assumption.

[5] Ibid.

Biological soil quality indictors: microbial community structure and biomass

Fungi, in particular, play an influential role in prairie ecology, and their presence can be an important indicator of ecosystem development. Certain mutualistic soil fungi form arbuscular mycorrhizal fungal (AMF) symbioses with the roots of prairie plants (Harnett and Wilson 1999). Many prairie plants have evolved to be dependent on these symbioses, which enhance plant nutrition and productivity; the thin AMF fungal hyphae are able to access scarce mineral substrates from tiny pores in the soil, which are unattainable by the relatively much larger roots of the host plant, and then transport those resources back to their hosts. The AMF symbiosis also influences the dynamics of plant competition, disease resistance, and ultimately plant community structure by differentially influencing plant growth and productivity (Bever et al. 2012, Harris, 2009). AMF are also important to the formation of soil structure (aggregation) and related ecosystem services, such as moisture retention and erosion resistance (Tisdall and Oades 1982, Beare 1997). It is also thought that fungi are composed of more recalcitrant compounds and might therefore promote whole-soil carbon (C) storage (Guggenberger 1999). In addition to harboring mutualistic AM fungal species, the prairie soils also contain saprotrophic fungi. These fungi utilize C substrates more efficiently than bacteria (Holland and Coleman 1987), and are unique in their ability to use and transport spatially separated nutrient resources (Frey 2003). Mature prairie systems are therefore considered fungal-dominated (Klein 1995, Ohtonen 1999, Williamson 2005).

The maturity and development of restored prairie systems can be indicated by the abundance of fungi in relation to bacteria, measured by the fungal: bacterial (F: B) gene copy ratio. In general, a low F: B (bacterial dominated community) occurs in agricultural soils where

nutrient loads are high and soil disturbances are common (DeVries et al. 2007). On the other hand, a high F: B is suggested to be indicative of more self-sustaining systems in which more pronounced N mineralization and decomposition of organic matter better provide resources for the plant community (Bardgett and McAlister 1999). Previous studies also support the significance of this indicator in carbon and nutrient cycling. For example, relatively high ratios of fungi to bacteria were found to correlate with increased organic matter decomposition and mineralization, as well as with decreased nutrient losses to the surrounding environment (deVries 2007). Higher F: B ratios have also been reported in extensively managed grassland, and associated with increased ecosystem efficiency (Sakamoto and Oba 1994) and food web complexity (Wardle 1995) in natural successional gradients in varying substrates. The mechanisms responsible for shifts in the soil microbial community remain largely unknown. Some studies have shown that soil management affects the F: B biomass ratio by altering the quantity and quality of root exudates, and changes in plant productivity, composition, and litter quality (Teague et al. 2011).

Total microbial biomass is also considered an important indicator of soil functioning, especially in regards to the maintenance of biodiversity (as producers at the bottom of a bacterial-based food web) and habitat provision (in creating micro-niche space; Gregorich et al. 1994, Sparling 1997). Additionally, previous work in grassland studies in northern France (Plassart et al. 2008) and the Chicago region (Allison et al. 2005) have demonstrated an increase in microbial biomass with prolonged restoration and management. One might expect increasing levels of microbial biomass to occur alongside increasingly prolonged restoration management in CWLMRP prairie sites, as well.

Physical and chemical soil quality indicators: aggregate structure, C, N, and pH

The scientific literature provides extensive evidence that F: B, and notably, the abundance of soil fungi, is related to soil structure (Jastrow 1998, Young et al. 1998, Six et al. 2004). For example, in a chronosequence study of restored grasslands in the Chicago region, scientists demonstrated a positive correlation between increasing F: B and increasing aggregate structure (Jastrow 1998). This result was attributed to the ability of fine roots and external fungal hyphae to act as binding agents and hold soil particles together to form macroaggregates (Jastrow 1998). Various studies have also found macroaggregate stability to be an important indicator of soil quality (Harris et al. 1996, Arshad et al. 1996, Karlen et al. 1996), particularly in its ability to sustain biodiversity, regulate soil hydrology, and contribute to physical soil structure and support for plants (Doran and Parkin 1994, Harris et al. 1996, Seybold 1998). Additionally, the formation of soil aggregates correlates with soil C carbon accumulation (Cambardella and Elliott 1993, Golchin et al. 1994, 1995, Besnard et al. 1996, Jastrow 1996, Angers et al. 1997, Franzluebbers and Arshad 1997; Monreal and Kodama 1997, Gale et al. 2000, Puget et al. 1995, 1996; Six et al. 1998, 1999, 2000, Paustian et al. 2000). It has been suggested that microaggregates (53- 212μm) are more recalcitrant and have a greater capacity to store C than macroaggregates (>212μm; Jastrow 1996). However, macroaggregate dynamics are critical to C sequestration, owing to greater encapsulation of C content in macro- than microaggregates (Jastrow et al. 1996), and because macroaggregates influence the formation and the level of C stored within microaggregates (Six et al. 2000b). When macroaggregates are destroyed, it decreases the formation of microaggregates within macroaggregates, and consequentially reduces C stabilization within those micro aggregates (Six et al. 1998, 1999, 2000).

Disturbance to prairie systems results in the degradation of soil aggregate structure and

its associated benefits. In both urban and suburban development processes, as well as large-scale agricultural practice, aggregate structure may be completely destroyed by the compaction caused by heavy equipment and machinery (Poesse 1992, Defossez 2002, Flowers and Lal 1998). In agricultural settings, the ability of plant roots to form and maintain aggregate structure may be weakened or destroyed by plowing (Unger 1994, Kadziene 2011). Therefore, I expect disturbed soils to demonstrate lower F: B ratios, aggregate abundance and C content than soils of highly developed and mature prairie systems.

Soil fertility, particularly levels of N and C, is also directly linked to microbial community structure and biomass (Bardgett 1993, Lovell 1995, Bardgett 1996, deVries 2007). For example, fertilization with inorganic nitrogen has been reported to reduce the F: B biomass ratio by reducing the abundance of soil fungi and stimulating bacterial biomass production (deVries et al. 2007). On the other hand, the addition of organic matter stimulates fungal growth to increase F: B. Soil acidity, as measured by pH, is also an important soil quality indicator (Doran and Parkin 1994, Smith and Doran 1996, Karlen et al. 1996) that can influence nutrient cycling, physical soil stability and soil hydrology (Doran and Parkin 1994, Harris et al. 1996, Daily et al. 1997, Seybold et al. 1998). However, the effect of soil pH on F: B appears to be context dependent. Changes in soil pH can have either a positive or a negative effect on F: B ratio (Rousk et al. 2009) depending on the differential responses of varying species within the bacterial and fungal communities to soil pH (Blagodatskaya and Anderson 1998, Rousk et al. 2010).

Other factors that might influence F: B include bulk density and texture (Larson and Pierce 1991, Doran and Parker 1994, Jastrow 1996). Thus, if an increased F: B ratio has a positive effect on plant nutrient uptake efficiency and nutrient retention, it is desirable to get a

better understanding of the management practices and soil properties that affect these factors as well.

Objectives and Hypotheses

The overarching objective of this study was to examine and evaluate the utility of various soil quality properties as markers of belowground recovery in a chronosequence of grassland restorations throughout the Chicago Wilderness region. I first investigated the belowground precision of land managers' vegetation-based determination of the restoration "sequence" by exploring the congruence between the belowground and aboveground systems in a management sequence. Next, I examined and compared the belowground systems with the land managers' vegetation-based determination of restoration "targets", by exploring the belowground congruence of pristine remnants and reference restoration. Finally, I investigated whether soil quality was higher in target prairies than in restorations along the management chronosequence to determine whether they represent true targets for whole prairie system recovery.

To do so, I examined and documented the impacts of prolonged land management on restored grassland soil properties, including both physical (texture, aggregation, bulk density), chemical (C, N, PO_4, pH, moisture) and biological (microbial biomass and microbial community structure) attributes, the latter represented, in this study, by the fungal: bacterial ratio. Soil factors were analyzed along a chronosequence of managed prairie restorations, and in target prairie sites, as previously delineated by the Chicago Wilderness Land Management Research Program. In turn, these soil factors were used to test the following three hypotheses:

H_1: Increasing duration of management will influence a shift towards a fungal-based soil food web, and enhanced aggregate structure and C storage (Figure 3).

H_2: Below ground microbial community structure of reference restorations will resemble that of pristine prairies (Figure 3).

H_3: Soil properties in pristine remnants and reference restorations will demonstrate lower fertility, and greater aggregate structure, C storage, and fungal abundance than restorations along the management chronosequence, and thereby provide belowground verification of the vegetation-based restoration targets (Figure 3).

Materials and methods

Study Design

This study used a "management chronosequence", in which the restoration sites had been maintained using similar management protocols but for varying amounts of time (Figure 4). This approach simulates a long-term study (Figure 5), by substituting space for time so that conclusions can be made about the short- and longer-term effects of restoration treatments. Sites in the chronosequence were classified as: 1) unmanaged (abandoned old fields, no restoration activities); 2) managed less than 10 years, and 3) more than 10 years. These sites were contrasted against pristine remnant prairie sites and reference prairie restorations, i.e., sites deemed by land managers to be restoration targets.

Site Selection

Fifteen mesic tallgrass prairies, spanning four Illinois counties in the Chicago region (Cook, Lake, McHenry, DuPage), were selected for this study (Figure 6). These prairies sites represent five different categories of restoration and management as defined by local land managers (Table 1). Within each category, sites were selected to minimize variation in soil texture and phosphorous content, as gleaned from the limited baseline soil data previously collected by CWLMRP scientists (Table 2). The five categories were as follows:

1) Abandoned and unmanaged old fields (abbreviated R0) that were reestablished on former agricultural land, but have never been actively managed (Hawk Hollow Bartlett Meadow, Hawk Hollow Northwest Prairie, Hawk Hollow Northeast Prairie).
2) Early-stage management prairie restorations (abbreviated R1) that were reestablished on former agricultural land, and have been managed for less than ten years (Bergman

Prairie, Smith Road Prairie, Spring Brook Prairie Central Field Bergman Prairie, Smith Road Prairie, Spring Brook Prairie Central Field).

3) Late-stage management prairie restorations (abbreviated R2) that were reestablished on former agricultural land, and have been managed for over ten years, under a prolonged-management regime (Spring Brook Prairie South Field, Half Day Road Prairie, Cuba Marsh).
4) Pristine prairie remnants (abbreviated P3) that were never plowed, and have been designated as "target" remnant ecosystems (Somme Prairie, Wadsworth Prairie, and Larson Prairie).
5) Reference prairie restorations (abbreviated R3) that were reestablished on former agricultural land, and have been designated "target" restoration ecosystems (Glacier Park Pioneer Road North Prairie, Glacier Park Pioneer Road South Prairie, Grant Woods).

Management Practices

The CWLMRP initiative conducts research on a network of natural areas that are managed by private contractors, non-profit organizations, government agencies and volunteers. Although prairies in this network, including all of the study sites selected for this study, each have unique, idiosyncratic histories of management, most follow a basic standard management regime, which includes seeding, burning, mowing, removal of trees, and spot-herbicide treatments (Kilde and Fuge 2002, USNRCS 2006, Taylor et al. 2009, MNNRCS 2012)[6].

[6] Although the Chicago Wilderness Land Management Research Program currently has few records on the exact history of each site, the information that is available on these sites is listed in Table 6 of the Appendix.

Soil Sampling and Collection

Soil cores were collected over the course of three weeks in mid-July, 2012 (see Table 3). July was one of the hottest and driest months of 2012, and no-to-little precipitation was recorded during the collection period. At each site, duplicate soil cores were collected with a tulip bulb planter (~10 cm diameter x 15 cm depth) and spade, from five separate locations: 1) the central GPS coordinates previously established by CWLMRP (Heneghan et al. 2012), and 2-5) 25 m away from the central GPS coordinate in each cardinal direction (Figure 7). Soil from each sample was processed immediately (KCl extraction and nutrient analysis, moisture, chloroform-fumigation analyses), air-dried for later analysis (texture, aggregate stability, pH, bulk density, total %C, %N), or stored at -80 ˚C (DNA extraction, amplification by qPCR). Soil for all analyses except bulk density was sieved to 8mm before further analysis.

Physical Analyses

Aggregate abundance

The abundance of water-stable macro aggregates and micro aggregates was determined by wet sieving (detailed in Elliot 1986). Air-dried soil was wet-sieved directly (allowing for slaking to occur) through a nested series of sieves (4mm, 2mm, 250μm, 53μm). Briefly, soil was placed on the 4mm sieve and the sieves were submersed in water and sieved vertically for ~2 minutes. The material collected on the each sieve was placed in aluminum foil pans, dried at 105˚C for two days, and weighed. Aggregates between 250μm- 2mm in diameter were defined as macro-aggregates (Tisdall and Oades 1982), while those between 53μm- 250mm in diameter were defined as micro-aggregates (Tisdall and Oades 1982).

Texture

Soil texture was defined as the percentage of sand, silt and clay in each sample, and was determined by deflocculating with 5% sodium hexametaphosphate and measuring sedimentation using a Bouyoucos hydrometer (Day 1965).

Bulk Density

Separate soil samples were collected at each sampling location for bulk density measurements using a tulip bulb planter (~10cm wide x 15cm deep). The volume of the soil collected was determined by lining the hole with plastic wrap, filling it with water from a graduated cylinder until level with surface, and noting the volume of water it took to fill the hole. The dry weight of these soils was weighed after two days in an oven at 105 ˚C. Bulk density was then calculated as dry weight (g) / water volume (cm^3) (NRCS 1999).

Chemical Analyses

Total carbon and nitrogen

Soil samples were oven-dried at 105ºC for at least 48 hours, and then ground into a fine homogenous powder, using a high-speed ball mill (Across International, Berkley Heights, NJ, USA). Duplicate samples were then packed into tin capsules (LECO Corporation, St. Joseph, MI, USA), and analyzed for %C and %N using a TruSpec CN Elemental Analyzer (LECO Corporation, St. Joseph, MI, USA). Prior to analysis, a subset of samples was tested for the presence of inorganic carbon, in the form of carbonates. Equal amounts of dry ground soil from each sample were loaded into a microplate, one drop of dilute (5%) hydrochloric acid was added to each well, and the presence of carbon dioxide gas bubbles was noted. None of the samples

tested showed bubbles, indicating the absence of inorganic C (Allison and Moodie 1965). Therefore, total soil C was considered representative of total soil organic matter.

Plant-available phosphorous, ammonium and nitrate

Dried and sieved oil samples were extracted with 1M potassium chloride using a 1:10 ratio soil: KCl, and then frozen at -20 ˚C until analysis with spectrophotometry. For each soil sample, two replicate sub-samples, were analyzed in duplicate.

Phosphate (PO_4) levels in individual KCl extracts were determined using an ammonium molybdate- malachite green reagent (Baykov et al. 1988), with samples read at 630nm on a microplate spectrophotometer (Epoch Microplate Spectrophotometer, BioTek, Winooski, VT, USA). Ammonium (NH4) levels were determined in KCl extracts using a salicylate reagent mixture (sodium salicylate, (tri)sodium citrate, sodium tartrate, sodium nitroprusside) and sodium hypochlorite solution, and the absorbance of the reaction product read at 650nm on the microplate spectrophotometer (Weatherburn 1967). Nitrate (NO3) levels were determined in KCl extracts using an acidic vanadium chloride- NEDD reagent mixture (Vanadium (III) chloride (VCl_3), sulfanilamide, **N**-(1-naphthyl)ethylenediamine dihydrochloride [NEDD]), and the absorbance of the reaction product read at 540nm (Doane and Horwath 2003).

Moisture

Soil moisture was determined gravimetrically. Duplicate 25g soil samples were weighed fresh from the field, oven dried to constant weight (105ºC for 48 hrs) and then reweighed. Percent gravimetric soil content was calculated as the difference between fresh and oven-dried soil samples as follows:

P = (([W] – [D])/ [D]) x 100

Where, P = percent (%) of total evaporable moisture content,

W = weight (g) of fresh soil, and

D = weight (g) of dry soil.

pH

For each sample, a 1:1 soil: deionized water slurry was mixed and allowed to equilibrate for at least 30 minutes at room temperature (22 ˚C). Soil pH was then determined by using a combination glass membrane electrode (Sartorius Mechatronics, Burt 2009)

Biological analyses

Microbial biomass

Microbial biomass was estimated by measuring the chloroform-labile N pool in the form of NO_3 and NH_4 relative to levels of NO3 and NH4 in non-fumigated soils (Hobbie 1998). Sub-samples of soil were fumigated with chloroform in a desiccator for 4-5 days and oven-dried (temp). Sub-samples of non-fumigated soil were also oven-dried, and then all samples were extracted and analyzed for NH4 and NO3 as described previously (see *Plant-Available phosphorous, ammonium and nitrate*). Microbial biomass N was then calculated as the difference between the sums of the fumigated NO_3 and NH_4 and the non-fumigated NO_3 and NH_4 as follows:

$N_B = EN/k_{EN}$

where N_B = microbial biomass nitrogen,

EN = chloroform-labile N pool

$EN = ([NO_{3Fumigated} + NH_{4Fumigated}] - [NO_{3Control} + NH_{4Control}])$,

and k_{EN} = a soil specific adjustment factor, estimated as 0.54 (Brookes et al. 1985)

Soil fungal: bacterial ratios

DNA extraction and quantification

DNA was extracted from individual soil cores using a PowerSoil DNA Isolation Kit (MO BIO, Inc., Carlsbad, CA, USA). One replicate was extracted according to the manufacturer's instructions. The second replicate was extracted using a modified procedure based on techniques taught at Michigan State University's Microbial Metagemomics Workshop (Instructors Schmidt, Waldron & Lennon). The modifications from this protocol only deviated slightly from the PowerSoil DNA Isolation Kit manufacturers instructions, and included an extra wash with high proof ethanol, an extra spin in a clean tube after the ethanol washes to improve the removal of ethanol before eluting the extract in ultrapure water, and heating the ultrapure water to 55C, prior to elution. Once isolated, DNA extracts were split into two $1/10^{th}$ diluted 100μl aliquots, and stored at -20°C until analysis.

DNA was quantified using a NanoDrop2000 UV-Vis Spectrophotometer (first replicate; Thermo Fisher Scientific, Wilmington, DE, USA) or a Qubit 2.0 Fluorometer (second replicate; Invitrogen Life Technologies, Carlsbad, CA, USA), using a Quibit dsDNA Broad Range Assay Kit), and processed according to the manufacturer's instructions.

qPCR

The relative abundance of fungi and bacteria was determined using quantitative polymerase chain reaction (qPCR) at the University of Illinois DNA Services Facility. Quantification of

bacterial small subunit rRNA genes (SSU rRNA) was performed as described previously (Nadkarni et al. 2002) using Taqman 2× Gene Expression Master Mix (Invitrogen, Foster City, CA). Primers and probes were synthesized and supplied by Integrated DNA Technologies (Coralville, IA, USA), with probe (ZEN double-quenched probe) and primer concentrations adjusted to identical levels.

Absolute quantification of bacteria was performed using a standard curve derived from PCR products generated by near-full gene amplification of SSU rRNA genes using the general bacterial primer set 27 F and 1492R (Lane, 1991). The standard curve was linear across a five order of magnitude scale (from 5.57E+06 to 5.57E+01 copies per reaction), with 90% efficiency (Figure 8a, 8b, and 9[7]). All soil unknown sample reactions were performed in duplicate and analyzed using an ABI 7900HT Fast Real-Time PCR system. Fungal SSU rRNA genes were amplified using the general fungal primer set EukA/B (Medlin et al. 1988). Subsequently, absolute quantification of fungal 18S rRNA genes was determined from the EukA/B primer product, using the primer set FR1/FF390 and the SYBR Green assay as described previously (Prévost-Bouré et al. 2011). The standard curve was derived from PCR product generated by amplification of 18S rRNA using the general fungal primer set FR1/FF390 and the SYBR Green assay as described previously (Prévost-Bouré et al. 2011). The fungal standard curve was linear across a four order of magnitude scale (from 1.05E+05 to 1.05E+01 copies/reaction), with 99% efficiency (See Figure 10a, 10b, 11 and 12). All unknown reactions were performed in duplicate and analyzed using an ABI 7900HT Fast Real-Time PCR system. DNA template-free control reactions (a.k.a no-template controls [NTC]) were also analyzed with each assay. Raw data, in

[7] There is no melt curve or melt peak associated with the bacterial assays, because they were conducted using a probe and primer pair.

the form of individual cycle threshold values (Ct) for each assay, was converted to copies/gram soil by the following steps:

1) Calculate the number of copies produced by the gene of interest in the standard genomic DNA: copy # = ([avogadro's number {6.02E23}] * [DNA concentration of undiluted standard {in ng/µl }] * [amount of template used for each qPCR reaction {2µl}]) / ([size of genome{1770 bp for fungi (Prévost-Buré et al. 2011, 466 bp for 27F/1492R bacteria (Nadkarni et al. 2002)}]*[average weight of a base pair of DNA {650g/mol}] * [number of copies in genome {default = 1 for both fungi and bactiera[8]}])

2) Calculate copy # for each dilution of genomic DNA (ie. Undiluted = x ng/µl; dilution factor of 1 = $x*10^{-1}$ng/µl, dilution factor of 2 = $x*10^{-2}$ ng/µl, …etc.)

3) Make standard curve graph (copy # on X-axis, Log_{10} of Ct value on Y-axis), to derive an equation for the line of best fit (y=mx+b; where x = copy # per reaction, and y =log_{10} Ct ; slope (m) should be negative). Rearrange equation to solve for copy # ($x = 10^{(b-y)}/m$).

4) Solve for copy # per reaction in all unknown samples that are run on the qPCR machine in the same run.

5) Calculate copies/g soil: (copy # / reaction)*(1 reaction / g soil) = copies/g soil)

[8] Because I did not know the exact number of copies of each targeted gene sequence in either the fungal or bacterial SSU genomes, I assumed 1 copy per genome. This may be inaccurate. Therefore, the absolute number of copies per reaction may not be valid. However, this may not be a problem for the purposes of this study as I am primarily interested in changes to the *relative* abundance of copies/g soil across management categories; I use this technique only to compare relative abundances of fungi and bacteria across site categories.

Reaction efficiency (E) represents the amount of PCR product increase after each cycle (ideal range: 90-100%), and was calculated from the slope of my standard curves, as follows:

$E = 10^{(-1/slope)}$ or, for reaction efficiency percentage $\%E = 10^{(-1/slope)} * 100$.

Statistical Analyses

Four sets of statistical analyses were conducted: a) data exploration and general hypothesis testing, and tests addressing b) hypothesis 1, c) hypotheses 2, and d) hypothesis 3. Data was analyzed using a combination of Microsoft Excel version 14.3.9 (Microsoft Corporation, 2010), JMP version 10.0.2 (SAS Institute, Inc., 2012), R version 3.0.3 (R Core Team, 2014) and RStudio version 0.98.501 (RStudio Inc., 2014).

Data exploration

I used JMP to produce descriptive summary statistics, and to assess data normality and transformation requirements, for all soil variables. I then used R and Microsoft Excel to create barchart visualizations of the impact of management on all soil variables.

Hypothesis 1

Linear-Mixed Effects Modeling: Quantification of Microbial Community Structure and Abundance Across the Management Chronosequence

Hypothesis 1 postulates that the microbial community structure and abundance shifts towards a greater fungal dominance with increasing duration of management. To test the influence of management duration on the microbial community structure, I used R and *lme4* (Bates, Maechler, Bolker and Walker, 2014) to perform a linear mixed effects analysis of the relationship between soil F: B gene copy ratios and the duration of management (R0, R1, and R2 only) using restricted maximum likelihood (REML) test criteria. I used the same method and test criteria to assess the influence of management on microbial community abundance. As a fixed effect, I entered management stage along the chronosequence for both F: B analyses and microbial biomass analyses. As random effects, I used intercepts for site, as well as by-site random slopes for the effect of the duration of management for both F: B gene copy ratios and microbial biomass-N. For both analyses, visual inspection of the residual histogram suggested a slight positive skew, but no major deviations from normality. P-values were obtained by restricted maximum likelihood (REML) ratio tests of the full model with the effect in question against the null model without the effect in question. Pair-wise comparisons between site types were determined with a one-way analysis of variance (ANOVA) and subsequent Tukey's Honestly Significant Difference (HSD) post-hoc tests, using JMP.

Ordination analysis: Assessing the Influence of Management Category and Abiotic Soil Properties on the Soil Microbial Community Structure and Abundance

To explore the relationship between the microbial community structure and other abiotic soil properties, in both managed and pristine prairies, I ordinated the biological soil sample components (fungal and bacterial gene copy numbers and microbial biomass) using non-metric

multidimensional scaling (NMDS), in R and *vegan* (Oksanen, 2014). NMDS is a nonlinear dimensionality reduction technique, commonly used in community ecology that entails robust and unconstrained ordination of sites. In this analysis, the ordination was created using Bray-Curtis dissimilarity distances (McCune and Grace 2002). Both fungal and bacterial gene copy number and microbial biomass data were Wisconsin square root transformed during the ordination analysis. Scaling effects include centering, PC rotation, and halfchange scaling. Additionally, I used a permutational analysis of variance (PERMANOVA), with 10,000 permutations to determine the significance of the ordination fit (i.e., stress). Physical and chemical soil properties, including % sand, % silt, % clay, ratio of macroaggregate to microaggregate abundance, %C, %N, bulk density, gravimetric moisture, soil phosphate, and pH, were fit onto the ordination chart as vectors to determine the relative influence of these variables on the ordination arrangement. R^2-values, p-values and vector coordinates were used to determine the fit, significance, and relative influence on NMDS axes 1 and 2 of each soil variable.

Multiple Regression Analyses: Correlations Between Microbial Community Structure and Abiotic Soil Properties across the Management Chronosequence

The relationships between the microbial community and other important soil factors along the management sequence were investigated using multiple regression analyses to fit both F: B gene copy ratios and microbial biomass nitrogen values to soil variables that were found to significantly influence the arrangement of soil samples in NMDS ordination space (C, N, pH, sand, silt, moisture). Because only prairie sites within the management chronosequence (R0, R1,

R2) give insight into the influence of management on these relationships, only chronosequence prairies were considered for these regression analyses. Additionally, carbon and nitrogen values were combined into one carbon: nitrogen (C:N) ratio.

Hypothesis 2

Linear Mixed-Effects Modeling: Assessing The Difference in Microbial Community Structure and Abundance Between Reference Restoration and Pristine Remnant Prairie Targets

Hypothesis 2 postulates that the microbial community structure and abundance in reference restorations will resemble those found in pristine prairies. To test this hypothesis, I used R and *lme4* to perform a linear mixed effects analysis of the relationship between soil F: B gene copy ratios and target prairie type (R3 and P3 only), using restricted maximum likelihood (REML) test criteria. I used the same method and test criteria to assess the influence of management on microbial community abundance. As a fixed effect, I used target category (e.g., R3, P3) for both F: B analyses and microbial biomass analyses. As random effects, I used by-site random slopes for the effect of target category for both F: B gene copy ratios and microbial biomass-N. For both analyses, a visual inspection of the residual histogram suggested slight positive skews and minor deviations from normality. P-values were obtained by restricted maximum likelihood (REML) ratio tests of the full model with the effect in question against the null model without the effect in question. A comparison between target site types was determined with a one-way analysis of variance (ANOVA) and subsequent Student's t post-hoc test, using JMP.

Hypothesis 3

Linear Mixed-Effects Modeling: Assessing the Trajectory of Management-Induced Shifts in Microbial Community Structure and Abundance in Relation to Target Prairies

Hypothesis 3 postulates that the microbial community structure and abundance in the management sequence (R0, R1, R2) demonstrates a shift towards that found in reference/pristine prairie targets (R3 and P3). To investigate the trajectory of changes to the microbial community structure and abundance, in managed and target prairies, I used R and *lme4* to perform linear mixed effects analyses of the relationships between site category and both soil fungi:bacteira qPCR ratios and microbial biomass-N. As a fixed effect, I entered site category. As random effects, I used intercepts for site, as well as by-site random slopes for the effect of site category. Visual inspection of a residual histogram and Q-Q plot suggest a slight positive skew, but no major deviation from normality. P-values were obtained by restricted maximum likelihood (REML) ratio tests of the full model with the effect in question against the null model without the effect in question. Pair-wise comparisons between site types were determined with a one-way analysis of variance (ANOVA) and subsequent Tukey's Honestly Significant Difference (HSD) post-hoc tests, using JMP.

Principle Component and Multiple Regression Analyses: Correlations Between Microbial Community Structure and Abiotic Soil Properties in Managed and Target Prairies.

To further determine the role of physical and chemical soil properties in assembling microbial community structure in all managed and target sites, I performed a principle component analysis (PCA), in R, using the NMDS-significant environmental vectors (carbon, nitrogen, pH, sand, silt, moisture; Table 5), after which I used multiple regression analyses to fit the first and second principle component axis scores to both F: B gene copy ratios and measures of microbial biomass-N, using both JMP and Microsoft Excel.

Results

Quantification of soil fungal: bacterial (F: B) gene copy ratios

Interpreted from relative copy number alone (Table 4), the duration of management significantly affected the F: B gene copy ratios ($\chi^2(2) = 24.251$, $p = 5.42e\text{-}06$; Figure 13). The F: B gene copy ratios were lowest in unmanaged sites and increased significantly after restoration and management. However, the difference in F: B between early (R1) and prolonged management (R2) was not significant ($P > 0.05$). The observed increase in F: B gene ratios along the management chronosequence (R0→R1→R2) was due to a increase in fungal gene copy numbers coupled with a dramatic decrease in bacterial copy numbers (Table 4).

Fungal: bacterial gene copy ratios in pristine remnants (P3) did not significantly differ from those in reference restorations (R3; $F(1) = 0.065$, $p = 0.812$). While pristine remnants had greater mean fungal gene copies than reference restorations (Table 4), the variability of this measure within pristine prairies was so large that it negated any significant effects between the two site categories.

When compared across all management categories, the F: B gene copy ratio was highest with prolonged management and lowest in unmanaged sites, reference restorations and pristine remnants (Figure 13). Nevertheless, the highest gene copy numbers for fungi still occurred in the pristine remnants (Table 4).

Soil microbial biomass nitrogen

Observations of microbial biomass nitrogen neither differed along the management chronosequence ($F(2) = 0.924$, $p = 0.447$), between the target prairie types ($F(1) = 0.004$, $p = 0.952$), or between all chronosequence and target sites combined ($\chi^2(4) = 7.516$, $p = 0.111$;

Figure 14). Microbial biomass nitrogen across all site categories averaged 481 ± 16 μg g^{-1} soil (mean ± 1 s.e.). Although there were no statistically significant differences in microbial biomass N among categories, the mean microbial biomass shows a decreasing trend with increasing duration of management (R0→R1→R2). In addition, the largest microbial biomass occurred in unmanaged sites and the lowest in target sites, particularly in pristine prairies, suggesting at least a trajectory in the chronosequence towards target levels. However, the large variations in biomass, particularly in early-stage and prolonged management restorations, prevented a significant treatment effect.

Microbial community composition among management categories and in relation to soil physical and chemical variables

The non-metric multidimensional scaling (NMDS) ordination of fungal gene copy number, bacterial gene copy number, and microbial biomass nitrogen in the management chronosequence and reference and remnant prairie produced a two-dimensional ordination with low stress (final stress = 0.0358, $R^2 = 0.516$; Figure 15). Based on NMDS analysis and a PERMANOVA, management categories could be separated into three broadly clustered areas in ordination space ($P < 0.05$) as follows: A) unmanaged prairie restorations (R0), B) prairie restorations in early-stage and prolonged management (R1 and R2 respectively), and C) reference restorations and pristine prairies (R3 and P3 respectively; Figure 15). The overlap between reference restorations (R3) and pristine prairies (P3) indicates similarity in biological community structure in both target prairie types. Additionally, there was no significant difference between managed prairie types (R1, R2), which is consistent with the similarity in fungal gene counts (Table 4) and F: B between these sites (Figure 15). However, pair-wise comparisons

showed that unmanaged sites (R0) were significantly different from all other sites, and that restored sites (R1, R2) were significantly different from target sites (R3, P3).

These patterns may be explained by a number of physical and chemical soil factors. Vectors of the environmental variables showing the most significant correlation ($P < 0.05$; Table 5) with site arrangement within the ordination appeared to form gradients parallel to axis 1 and axis 2, and included both physical and chemical soil factors. Environmental variables that displayed a negative correlation with axis 1 include those related to moisture availability. Thus, significant differences in unmanaged sites from restored and target sites were likely generated by differences in soil moisture availability. In contrast, variables showing a positive correlation with axis 1 (vectors overlaying the reference restorations and pristine prairies) were total % N, C, pH and % sand. Thus environmental markers of restoration targets include increases in soil total % N, % C (organic matter), and pH (Figures 16, 17, 18). Variables showing significant positive correlation with axis 2 (vector associated with early, prolonged management) was % silt. Increasing soil % silt might thus explain much of the difference between early and prolonged management (R1, R2) and target prairies (R3, P3). The inclusion of both % sand and % silt as significant environmental variables underscores the importance of texture in restoration and management. However, there was no significant effect of bulk density, available phosphate, % clay, or the abundance of macroaggregates relative microaggregates on the NMDS ordination arrangement. That aggregate abundances were not directly correlated with the NMDS ordination suggests that soil structure is indirectly influenced by management.

Correlations between the microbial community[9] and soil physical[10] and chemical[11] variables

The relationship between the shifting microbial structure (F: B, biomass) and significant soil factors identified in the NMDS was gleaned using multiple regression analyses. Only chronosequence prairies were considered for regression analyses because sites within the management chronosequence (R0, R1, R2) can provide insights into the cumulative effects of management.

A significant positive correlation was found between F: B gene copy ratios and soil C: N ratios ($R^2 = 0.1629$, $p = 0.006$, Figure 19), suggesting that management-induced shifts in the microbial community structure of restored prairie could occur within aggrading systems. Significant correlations were also found between F: B gene copy ratios and % sand ($R^2 = 0.1839$, $p = 0.003$, Figure 20) and % silt ($R^2 = 0.1924$, $p = 0.003$, Figure 21), indicating that changes in the relative amounts of these soil fractions among sites are likely to have significant effects of fungal and bacterial abundances.

A significant negative correlation was detected between gravimetric soil moisture and F: B gene copy ratio ($R^2 = 0.1608$, $p = 0.006$, Figure 22). In contrast, a significant positive correlation was detected between gravimetric soil moisture and microbial biomass nitrogen ($R^2 = 0.3439$, $p < 0.0001$, Figure 23). These findings suggest that increasing moisture plays a role in shaping microbial community structure by enhancing the abundance of bacteria. On the other hand, the decline in F: B gene copy with loss of soil moisture suggests that fungi may become more dominant with decreasing soils moisture.

[9] Biological soil property data depicted in Appendix Table 7.
[10] Physical soil quality data depicted in Figure 24 and Appendix Tables 8 and 9.
[11] Chemical soil quality data depicted in Appendix Tables 8 and 10.

Variance of physical and chemical soil variables in relation to soil microbial community indicators

Principal component analysis was used to calculate new synthetic variables (principal components) that were linear combinations of the original environmental variables, and reduce the multidimensional data set down into two axes that contained the maximum amount of variation from the original data set. Based on the loadings, PCA1 (55.2%) primarily explained variations in soil fertility (C, N, pH), whereas the variation in PCA2 (25.8%) accounted for physical attributes such as texture (sand, silt) and gravimetric moisture.

Linear regression analysis revealed an inverse correlation between F: B gene copy ratio and PCA1 axis scores ($R^2 = 0.1665$, $p = 0.0003$; Figure 25), thereby highlighting the role of soil fertility in assembling microbial community structure. Specifically, the negative relationship between PCA1 and F: B gene copy ratio suggests that the microbial community structure may shifts towards a more fungal-based soil food web with decreasing soil fertility. Linear regression analysis also revealed an inverse correlation between PCA2 axis scores and microbial biomass nitrogen ($R^2 = 0.4225$, $p < 0.0001$; Figure 26), thereby highlighting the important role of soil texture, and its ability to modulate soil moisture, in determining microbial abundance. However, there was no statistically significant relationship between F: B gene copy ratio and PCA2 axis scores (Figure 27) nor between microbial biomass nitrogen and PCA1 axis scores (Figure 28).

Discussion

The primary goal of this project was to assess the impacts of a long-term general management regime on soil recovery in prairie restorations around the Chicago Wilderness area, and to determine whether measurements of any single soil quality component could be used as a surrogate indicator for whole soil health. Overall, the study suggests that prolonged management effects a shift in microbial community structure towards a more fungal-based soil food web, and that this shift occurs simultaneously with changes in soil fertility and variation in other edaphic properties. Additionally, the study suggests that the soil fungal: bacterial (F: B) gene copy ratio may be a valuable indicator of the maturity and development of early and mid-stage prairie restoration. Further, the belowground similarity in microbial community structure between high quality restoration and remnant prairies supports the precision of land managers' designations of prairies as "targets" for restoration. However, the trend for gross microbial community structure to move away from target prairies, with increasing duration of management, suggests the F: B gene copy ratio may loose some value, as a sole indicator of prairie maturity, in prairies on the very high end of the spectrum of maturity and development. Adequate evaluation of highly advanced prairie restoration may require other indicators that can give more granular resolution of the microbial community composition.

Hypothesis 1 – The Management Chronosequence

The significant increase in fungal gene copy reads and increasing F: B ratio between unmanaged old fields (R0), restorations in the early-stages of management (R1), and restorations under more prolonged management (R2) support the hypothesis that prolonged management produces a shift in the microbial community from a bacterial dominated (R0) to an increasingly

fungal-based soil food web (R1, R2). This result suggests the F: B ratio may be a useful indicator of restoration efforts. The observed increase in F: B is consistent with observations made in a restoration chronosequence of ex-arable grasslands within the Chicago region where fungal biomass (based on phospholipid fatty-acid analysis [PLFA]) exhibited an initial increase in the first sixteen years of restoration (Allison 2005), and with the abundance of fungal biomass (derived from microscopy counts) observed in a chronosequence study conducted in ex-arable organic dairy farm pastures in the central part of the Netherlands (van der Wal 2006). Further, a chronosequence study conducted in ex-arable semi-arid shortgrass steppe lands of the Colorado Central Plains (USA), also found a higher fungal biomass in older fields (Klein 1995).

This finding also suggests an increasing role for fungi in restored systems. The fungi in these systems likely comprise both saprobes and mutualists. The results from this study do not allow me to differentiate between these two functional groups. However, it is possible to hypothesize that the large differences in edaphic conditions among the chronosequence sites likely altered the abundance of saprobes and mutualistic fungi. For instance, saprotrophic fungi acquire resources directly from the soil environment, and are therefore more sensitive to changes in soil C, N, and moisture than are mutualists. If so, the increase in fungi along the management sequence may represent an increase in mycorrhizal fungi, particularly the Glomeromycota, which are known to increase in abundance with increasing length of prairie restoration (Jastrow 1987, 1996).

The increase of fungi in relation to bacteria was also correlated with measures of soil fertility, notably soil C: N and total N. The changes in F: B ratio with C: N is consistent with theories of fungal C efficiency (Six et al. 2006), and with studies in which shifts in the abundance and structure of microbial community are attributed to quantitative and qualitative

changes in organic substrates (Holland and Coleman 1987, Yeates 1997), and soil C availability (Jastrow 1987, 1996, Frostegård and Bååth 1996, Allison 2005, deVries 2007, Pennanen 2011). Likewise, the increase in the F: B ratio with decreases in soil N is consistent with results from grasslands studies conducted across the Netherlands (deVries 2007) and in South West England (Bardgett 1993, Lovell 1995, Bardgett 1996), in which F: B ratios were highest in unfertilized grassland soils, and lowest in soils with high levels of N-based fertilization. Further, my findings are consistent with a study conducted on both mesic prairie and semi-arid shortgrass steppe in the United States, in which soil N was found to be a major driver of fungal abundance and community structure, specifically that of Glomeromycota fungi (Egerton-Warburton et al. 2007). The decrease in N, along the management chronosequence, may reflect changes primarily to synthetic nitrogen, rather than organic N sources, as the legacy of agricultural fertilizer application (i.e., anhydrous ammonia, ammonium nitrate, and other man-made fertilizers) subsides.

It is important to note that, despite the wide variations in physical and chemical soil characteristics across the management sequence, shifts in F: B among management categories were linked to specific edaphic properties. Land-use management has been shown to influence the physical structure and chemical composition of the soil, and the structure of the microbial community (Boddington and Dodd 2000; Girvan et al. 2003; Johnson et al. 2003), and the importance of edaphic factors in shaping microbial communities have been established by a number of studies (Bååth and Anderson 2003).

In unmanaged sites (R0), the driver was soil moisture whereas in early and prolonged management sites (R1, R2), there appeared to be a strong link between F: B and % silt. The strong relationship between F: B and soil moisture in the unmanaged sites was not surprising

since moisture availability can strongly affect microbial activity by limiting substrate diffusivity and accessibility (Bachar et al. 2010, Drenovsky et al. 2010, Blankinship et al. 2011). That a low F: B also correlated with low soil C: N suggests that moisture and substrate quality enhanced microbial (bacterial) activity and growth, and the turnover of soil organic C in unmanaged sites (Parton et al. 2007, Dequiedt et al. 2011). The correlation between F: B gene copy ratio and % silt in early and prolonged management sites is broadly consistent with work by Girvan et al. (2003) in which soils with the same texture contained similar microbial communities. Soil texture was shown to influence soil fungal community structure in agricultural fields of Zimbabwe (Lekberg et al. 2007) by influencing the ability of species to grow through the soil matrix (Jakobsen et al. 1992, Smith and Read 2008). Further, previous work found that microbial community variation is closely linked to the relationship between soil texture and moisture availability owing to microbial niche partitioning (Meobius-Clune et al. 2012). Silt is an intermediate-size soil particle, holding more water than sand, but less water than clay and may therefore stabilize fluctuations in soil water potential. Together, these results suggest that the F: B gene copy ratio in the restoration chronosequence may have been determined by the underlying soil chemistry and structure, in addition to the duration of management.

Hypotheses 2 and 3 – Restoration Targets and Management Trajectory in Relation to Targets

The finding of a similar mean F: B gene copy ratio between reference restorations (R3) and pristine prairies (P3) supports the second hypothesis, which predicts a similar resemblance between the biological communities of target prairies (R3 and P3). These results affirm the *precision* of land managers' designations of prairies as targets for restoration. There are few published studies to date that examine restoration goals and the adequacy of land managers'

designations of references for restoration work[12]. Land managers largely identify reference restorations based on their aboveground floral characteristics. The designation of R3 and P3 prairies as targets suggests they share similar vegetative and other aboveground characteristics that are desirable to land managers (see Figures 29 and 30). The demonstrated belowground similarity between target sites in this study is consistent with an abundance of evidence demonstrating the close relationship between aboveground and belowground biota (see Wardle et al. 2004, Bardgett et al. 2005, De Deyn and Van der Putten 2005, Voegelsang 2006, van der Putten 2009). Nevertheless, evidence exists to suggest that variations in the fungal community composition are more strongly related to differences between sites than to similarities in the vegetation aboveground (Avis et al. 2008). Taken together, these findings hint that the relationship between aboveground and belowground biota may become more complex and nuanced when considering more fine-scale resolutions of the microbial community.

The drivers of F: B in reference restorations and pristine remnants comprised soil N, C, and pH, whereby relatively high levels of soil N, C and pH (PCA 1) were correlated with relatively low F: B ratios. The relatively high N found in the target prairies may be attributed to a gradual buildup of a more recalcitrant, organic, and slow cycling pool of nitrogen (Yermakov and Rothstein 2006), such as that derived from prairie legumes and fungal chitin. Likewise, relatively high C levels may reflect vegetation build-up in these highly developed and mature prairies (Yermakov and Rothstein 2006). Finally, it is possible that pristine target prairies exhibit higher levels of pH because they do not have the same, or as recent, history of agricultural treatments with acid-forming synthetic fertilizers (Wortmann et al. 2003), as do the

[12] But see Matthews and Spyreas (2010) and Emery and Rudgers (2010).

managed restorations. Therefore, soils in old fields and less developed restorations may be more acidic, due to their agricultural legacy, than soils in highly developed pristine remnant prairies that have never been treated with such fertilizers. This could explain the difference in pH driving the separation between managed and target sites, in ordination space.

While these results supporting the second hypothesis suggest close aboveground-belowground relationships, and support the *precision* of land managers' "target" designations, there was less evidence to support the third hypothesis, which predicted that the biological communities in restored prairies (R0, R1 and R2) converges with those of target prairies (R3 and P3) after prolonged management. Based on the *fungal gene copy number alone*, there is a trend for fungal abundance along the management chronosequence to increase towards levels found in pristine remnants (P3), and away from the lower levels found in reference restorations (R3). This trend hints that remnant prairies (P3) may be more accurate than reference restorations (R3) in their representation of whole-system targets. Despite this trend, F: B ratios initially increased with increasing duration of management, and then declined between prolonged management (R2) and both reference restorations (R3) and pristine remnants (P3). This result suggests that non-linear responses to restoration occur belowground and, as a result, there may be incongruence between the trajectory of belowground soil recovery and aboveground, vegetation-based targets for restoration outcomes.

This possibility raises questions about the *accuracy* of choosing whole-prairie "targets" based solely on their aboveground characteristics. However, it is possible that the relatively low F: B ratio and microbial biomass seen in target prairies may be masking a more diverse, yet less abundant, fungal community structure. Thus, the increase in F: B seen in the management chronosequence (R0→R1→R2) may be context dependent. It is also possible that the

management chronosequence does not currently include grasslands with a sufficiently wide range of restoration ages so as to be comparable with reference restoration or pristine remnant prairies. Further, there may be succession within the microbial communities – from early- to conservative late-successional taxa – that influences community characteristics based on the life history and competition strategies of the individual species.

Unexpected Results

Surprisingly, shifts in F: B among management categories were not significantly linked to the abundance of water-stable macroaggregates or microaggregates, or to their relative relationship to eachother. In fact, there was no significant difference in the abundance of aggregates among sites. This was unexpected because previous work has suggested that soil aggregation increases with restoration age, and that a greater fungal abundance, particularly of the Glomeromycota, promotes the buildup of macroaggregates and the stabilization of aggregate structure (Miller and Jastrow 1990, 1992, 2000, Jastrow 1998, Zhu and Miller 2003, Rillig and Mummey 2006). It is possible that aggregate abundance is indirectly related to microbial community structure, whereas other factors, such as fertility, are more clearly tied to F: B and/or mask the importance of soil aggregates in the NMDS analysis.

It is also possible that the community composition of species within Kingdom Fungi may influence macroaggregate stabilization. Different fungal taxa vary in their capabilities to radiate hyphae throughout the soil matrix (Abbot and Robson 1984), and therefore in their ability to bind soil particles into aggregates, and promote the process of soil stabilization (Rillig and Mummey 2006). While it is well documented that the hyphae of mutualistic mycorrhizal fungi help to stabilize macroaggregate structure (Miller and Jastrow 1990, 1992, 2000, Jastrow 1998, Zhu and

Miller 2003, Rillig and Mummey 2006), I have found little evidence in the literature to suggest that saprotrophic fungi work to similarly build aggregate structure. Unlike mycorrhizal fungi, saprotrophic fungi do not grow into and around living prairie plant roots, but rather grow through dead organic material. Therefore, they may not work in conjunction with plant roots, in the way that mycorrhizal fungi do, to effectively build aggregate structure. Such difference in fungal growth strategies may lead to differences in aggregate formation in soils that harbor differing proportions of saprotrophic versus mycorrhizal fungi. Likewise, more fine-scale differences in composition of the mycorrhizal fungi phylum, *Glomeromycota,* may also differentially influence macroaggregate structure formation. For example, mycorrhizal fungi that have greater proliferation of extra radical hyphae, such as *Gigasporaceae* (in cm/g soil; Abbott and Robson 1985), might better wrap around soil particles to form macroaggregate structure, than their phylogenetic neighbors with less prolific extra radical hyphae, such as *Glomeraceae* (Abbott and Robson 1985).

Soil texture may also be masking any relationship between F:B and macroaggregate stabilization. This possibility is supported by evidence from the literature that suggests texture influences aggregate formation (Bronick and Lal 2005). For example, soil organic carbon seems to play a bigger role in aggregate formation in coarse-textured sandy soils than in more fine-textured clay soils. Varying types of soil clays also differ in their capability to form aggregate structure (Bronick and Lal 2005). Thus, differences between my sites, in both soil texture and fungal community composition, may have confounded my aggregate data. Future study to decipher the relative contributions of both of these elements to my aggregate data might help clarify the relationship between microbial community structure and macroaggregate stabilization.

Unexpectedly, soil P was also not significantly linked to the NMDS-dispersion of sites according to F: B gene copy ratio. Soil phosphate is an important and often limiting plant nutrient, and many tallgrass species form important symbiotic relationships with members of the Glomeromycota so as to acquire P in exchange for C. In addition, bacterial and fungal community abundance can change with soil P content as conditions may enhance or suppress the proliferation and activity of specific microbial taxa. Although prairies were selected to minimize variation in P *within* site types, I expected to see P vary inversely with F: B *across* site types, as plants depleted any residual P from agricultural fertilizers over time. At first glance, it would appear that these results suggest that soil P fertility might not influence processes involving soil microbes. However, the soil samples for this study were collected on one occasion (summer 2012). Thus, there is the possibility that seasonal changes in plant phenology, and their affect on soil P availability and cycling, might be better correlated with microbial abundance and F: B than soil P alone, as has been shown in other studies (e.g., Beauregard et al. 2010).

Also puzzling was the slightly higher, though not statistically significant, level of P found in target prairies. Previous work has shown that P adsorbs particularly strongly to the glacial-loess derived soils in Illinois with the result that soil P is inherently higher in Illinois than other Midwestern states (Kurtz et al. 1945). It may be that the legacy of disturbance in restored prairies has influenced their levels of soil P.

Non-significant factors

Although the duration of management did not appear to significantly influence microbial abundance, increases in soil moisture within the chronosequence were associated with higher levels microbial biomass-N. This finding is consistent with grassland research on a fertility

gradient in the southwest of England, which found decreases in microbial biomass-N with increasing drainage (Bardgett 1999). On the other hand, I found that soil fertility, as measured by the component of variation composed of C, N, and pH, had no influence on microbial biomass-N. This finding is in contrast to results from the same study in the southwest of England, which found that microbial biomass-N increased following the application of relatively large quantities of N-based fertilizer (Bardgett 1999). Although microbial biomass observations do suggest a decreasing trajectory through the chronosequence towards target levels, the effect was insignificant; there was no evidence to support that microbial biomass-N is a useful indicator of prairie soil recovery.

Bulk density also did not appear to determine the biological differences between site categories, nor did any patterns emerge in bulk density observations within the management chronosequence, between target prairie types, or across all site categories. These findings are in contrast to the results of a similar study in the Chicago region, which found bulk density greater in pristine prairies than in early-stage restorations (McKinley 2005), and studies where both texture and bulk density were shown to modify F: B and microbial biomass (Bach et al. 2010). Future studies that tease apart the relative importance of different soil factors would help to clarify these possibilities.

Limitations

There are some limitations in exclusively considering the abundance of fungi in relation to bacteria when evaluating ecological restoration. For example, the F: B ratio indicator of the soil microbial community does not provide insights into the diversity and community structure within the broader kingdom fungi nor within the domain bacteria. Therefore, this study cannot

preclude the possibility that differences seen between the management categories are influenced by more specific nuances in their fungal and bacterial communities. For example, it is possible that sites with high fungal abundance may contain more weedy or pathogenic fungi, or that sites with lower fungal abundance could contain more conservative and diverse fungal species, with unknown effects on F: B ratios. Additionally, our methods cannot differentiate between prairies containing conservative and weedy taxa within the phylum of mutualistic prairie fungi, Glomeromycota. Such factors may limit the conclusions that can be drawn from F: B gene copy ratio. The development and use of more specific fungal primers, such as those that capture the abundance of the Glomeromycota and the conservative *Gigasporaceae* family, in particular, will help to more accurately assess prairie soil recovery. Concurrent work is underway to develop these tools (Palmer et al. *in progress*). With such tools, both the relative qPCR abundance of general Glomeromycota and conservative taxa within the phylum could be compared to the F: B gene copy ratios to help detangle differences between prairie categories, and would thereby enhance the utility of the F: B gene copy ratio as a marker of restoration development and maturity.

There are also technical limitations to measuring the abundance of fungi in relation to bacteria in the soil using qPCR. Primers vary in their specificity and ability to amplify template DNA in different taxa (Diffenbach 1993), so qPCR results may not reflect the total diversity of soil microbial communities (Fierer et al. 2005). Poorly optimized primers may preferentially amplify certain taxa in a group, and thereby misrepresent the absolute abundance of the group as a whole (Polz and Cavanaugh 1998). The ratio of extracted DNA template to the qPCR-amplified product may also be skewed by stochastic variation in the early stages of the qPCR reaction, termed PCR drift (Polz and Cavanaugh 1998). DNA extraction bias may lead to a

misrepresentation of the abundance of certain taxa, making it difficult to compare studies using different extraction methods (Anderson and Cairney 2003), and DNA extraction bias may lead to a misrepresentation of the abundance of certain taxa (Martin-Laurent 2001). Additionally, heterogeneity in rDNA copy numbers may lead to a misrepresentation of the true abundance of taxa. Molecular analyses of samples that pool an array of taxa, as environmental soil samples, are prone to skew estimations of community structure (Avis et al. 2010). If one sample is dominated by a species with a high rDNA copy number, and another sample is dominated by a species with a low rDNA copy number, the qPCR abundance will be different, even if the in situ abundance is exactly the same. In short, there is a high risk of false positives, in determining total abundance, if all taxa and their rDNA copy numbers are not known (Morgan, *personal comm*). Despite these limitations, there is an accumulation of evidence supporting the use of qPCR analysis of fungal and bacterial soil communities (Fierer et al. 2005, Prevost-Boure 2011, Gorzelak et al. 2012, Rousk et al. 2010, Nadkarni et al. 2012, Thonar et al. 2012, Strickland 2013). While qPCR is an indirect measure of fungal abundance, it is no different, in that regard, from other methods approximating fungal biomass, i.e., percent root colonization, fatty acid profiling, or ergosterol measurements (Gorzelak et al. 2012). Fungal and bacterial primers in this study were carefully designed and optimized (Prevost-Boure et al. 2012, Nadkarni et al. 2012), and had very high reaction efficiencies, lending support to the integrity of our findings. Our DNA extraction and template purification protocols were also highly standardized, which should have minimized any differential bias in extraction and amplification efficiency among our samples, and supported the comparability of our results. Finally, while our qPCR methodology does not allow us to determine *absolute* fungal abundance, for reasons stated above, this

potential limitation does not impact our study, as we only utilize *relative* fungal and bacterial gene copy numbers as they vary across prairie sites.

As in other chronosequence research, this study makes a space-for-time assumption (Allison et al. 2005, van der Wal et al. 2006, Yao et al. 2006, Zornoza et al. 2009, Vitousek et al. 2010). Conclusions about the impacts of management duration are drawn from prairies in separate geographical locations that have been managed for differing amounts of time. All of my prairie sites are located off the southwest coast of Lake Michigan, within the Chicago region, and I took efforts to minimize site-differences in nutrients and texture. However, it is possible that more fine-scale differences between the geographic locations of sites could confound trends due to time if there are higher-resolution geography-related site differences that result in varying spatial autocorrelation across the region. Further, the space-for-time assumption could be problematic if other differences between individual sites obfuscate trends due to time (Pickett 1989). Although all prairie sites were considered to be under the same management regime (seeding, burning, mowing, tree-removal and spot-herbicide application), this study is limited in our understanding of the idiosyncratic management histories of each prairie site. Additionally, we did not include a control for the age of abandoned fields and restored prairies, excluding management regime. Nor did we account for potential variations in management intensity. Therefore, this study cannot tease apart the relative influence of management intensity, management duration, and the number of years since the cessation of agricultural disturbance. Further investigation into the management history of CWLMRP prairies would enhance the ability to differentiate the relative influence of these restoration factors.

Conceptual Model Revisited[13]

The original conceptual model (Figure 1), on which my study was based, postulated a number of relationships between different soil quality properties, as follows:

1) F:B and C:N ratios were expected to be positively related. This was borne out in my results ($p=0.006$, $R^2=0.163$, Figure 19).
2) C:N and microbial biomass were expected to be positively correlated. This was not found to be the case; on the contrary, in this study, they were negatively correlated ($p=0.0005$, $R^2=0.155$, Figure 31)
3) Biomass and the macroaggregate:microaggregate ratio were expected to be positively related. This was also confirmed in my study ($p=0.003$, $R^2=0.110$, Figure 32).
4) A negative correlation was expected between pH and C:N. This was also found in my study ($p=0.04$, $R^2=0.056$, Figure 33).
5) A positive correlation was expected between F:B and macroaggregate:microaggregates. This realationship was not confirmed in my study, surprisingly (Figure 34).

My conceptual model further postulated that soil quality would be represented by groupings of certain physical, chemical, and biological soil components, typical of differing stages of prairie development and maturity, and that these groupings occur along a spectrum, ranging from very degraded sites on the one extreme, to highly developed and mature sites on

[13] See Figures 35 and 36.

the other (Figure 2). Specifically, it was postulated that higher levels of nitrogen, phosphate and bacteria would be found in more degraded soils, whereas higher levels of carbon macroaggregates and fungi would be found in more highly developed, mature and pristine soils. As expected, degraded old fields were found to have higher levels of nitrogen and bacteria, and pristine prairies had higher C (See Figure 36 and Appendix [Figures 43, 38 and 42]). However, unexpectedly, phosphate did not vary across the spectrum, and macroaggregate:microaggregate and the relative abundance of fungi were not indicative of pristine prairies (See Figure 36 and Appendix [Figures 41, 50 and 37]). This suggests that, at least in this study system, at the time of collection (summer, 2012), soil N-based fertility, C, and C:N ratios, and pH were more prominent markers on the spectrum of soil quality than were aggregate soil structure, biomass or phosphate. Additionally, F:B ratios were useful in determining soil quality for degraded and intermediate quality soil, but lost their utility, as a marker, on the pristine end of the spectrum (Figure 35 & 36).

Future Studies

While F: B appears to be a useful indicator of early and prolonged restoration efforts, other indicators of the fungal community structure, particularly of the mycorrhizal community structure, are needed to characterize very highly developed and mature prairie. Further investigations within the fungal phylum of mycorrhizal plant mutualists, Glomeromycota, would better illuminate the influence of current Chicago-region restoration management practice on prairie soil recovery. In addition, the relative contributions of synthetic and organic N fertilization, and anthropogenic N deposition to soil N fertility may need to be addressed to better understand N cycling in these restored and managed systems.

Additional investigation into the floral community and other aboveground characteristics of CWLMRP prairie sites could illuminate connections between the soil community and whole ecosystem recovery and further enhance the predictive power of the analyses. To this end, establishing a Floristic Quality Assessment (FQA) and linking the index to the soil community would help illuminate aboveground- belowground linkages in site recovery. This might entail assessments of floral diversity, particularly of keystone species, such as the deeply-rooted *Silphium laciniatum* (compass plant), as well as those species with high coefficients of conservatism, i.e, C-values between 7-10 (Swink and Wilhelm 1994), including some warm season (C4) grasses, such as *Sporobolus heterolepis* (prairie dropseed, C-value = 10), prairie-specific forbs, such as *Eryngium yuccifolium* (rattlesnake master, C-value = 8), and nitrogen-fixing legumes, such as *Baptisia alba* (white wild indigo, C-value = 8) and *Viola pedatifida* (prairie violet, C-value = 9).

Finally, various indices of soil quality have been developed by different organizations, primarily for agricultural soil systems (Andrews 2004, Karlan et al. 1997, 2011, Parr et al. 1992, Smith et al. 1993). However, indices of soil quality for prairie restoration, in general, and in the Chicago Wilderness, in particular, have not yet been developed. It may be useful to develop a soil quality index specific to prairie ecosystems, in general, and also for prairie restoration in the Chicago region, in particular. Developing such an index would entail establishing a range of ideal unit-value levels for each soil quality measurement, in Chicago-region prairie systems, as well as a weighted ranking for each soil quality component by its relative influence on whole-soil quality. This would allow scientists to assign index scores to their soil samples, and thereby compare samples using one, integrated, whole-soil metric, on a weighted, numerical soil quality index.

Conclusions

Overall, I found that the duration of management in Chicago Wilderness prairies influences the microbial community structure by increasing the abundance of fungi relative to bacteria, and that these microbial shifts occur with decreasing N fertility and variation in other edaphic properties. These data imply that management duration, at least in the first couple of decades, brings about a bottom-up trophic cascade, shifting the system towards a more fungal-based prairie food web. I propose that these trophic shifts may influence N-cycling and other ecosystem processes. My results also lead to the conclusion that the qPCR-derived F: B gene copy ratio is a valuable indicator of ecosystem development, particularly within the first one to two decades. However, to distinguish highly developed and mature prairie communities, other indicators, such as the abundance of conservative mycorrhizal taxa within the Glomeromycota, may be needed. Finally, this study makes one of the first attempts to evaluate aboveground-belowground linkages in the precision and accuracy of target prairies designations in the Chicago Wilderness Land Management Research Program (CWLMRP). My findings strongly support the close aboveground-belowground linkages in target prairies, and affirm the consistency of CWLMRP target prairie designations. While these results hold immediate significance for prairie restoration in the local Chicago Wilderness, I anticipate the relationships demonstrated between prairie restoration management, the soil food-web and N-cycling, may be informative for restoration work in other grassland systems, as well.

Figures

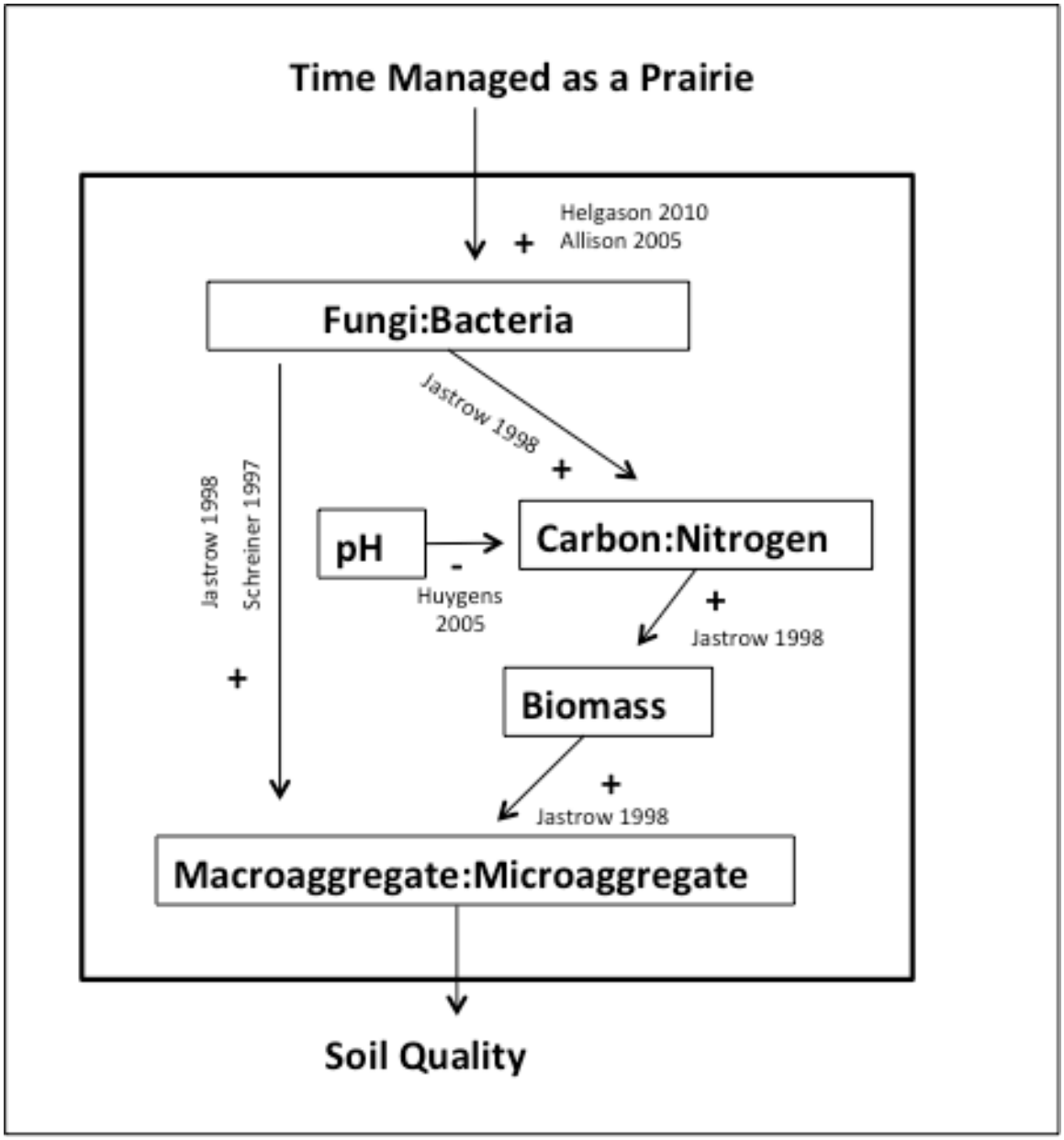

Figure 1. Soil Quality Constituent Interaction Model. Hypotheses propose that all components of the model (fungi:bacteria, carbon:nitrogen, macroaggreate:microaggregate, and microbial biomass), with the exception of pH, increase with increasing duration of management.

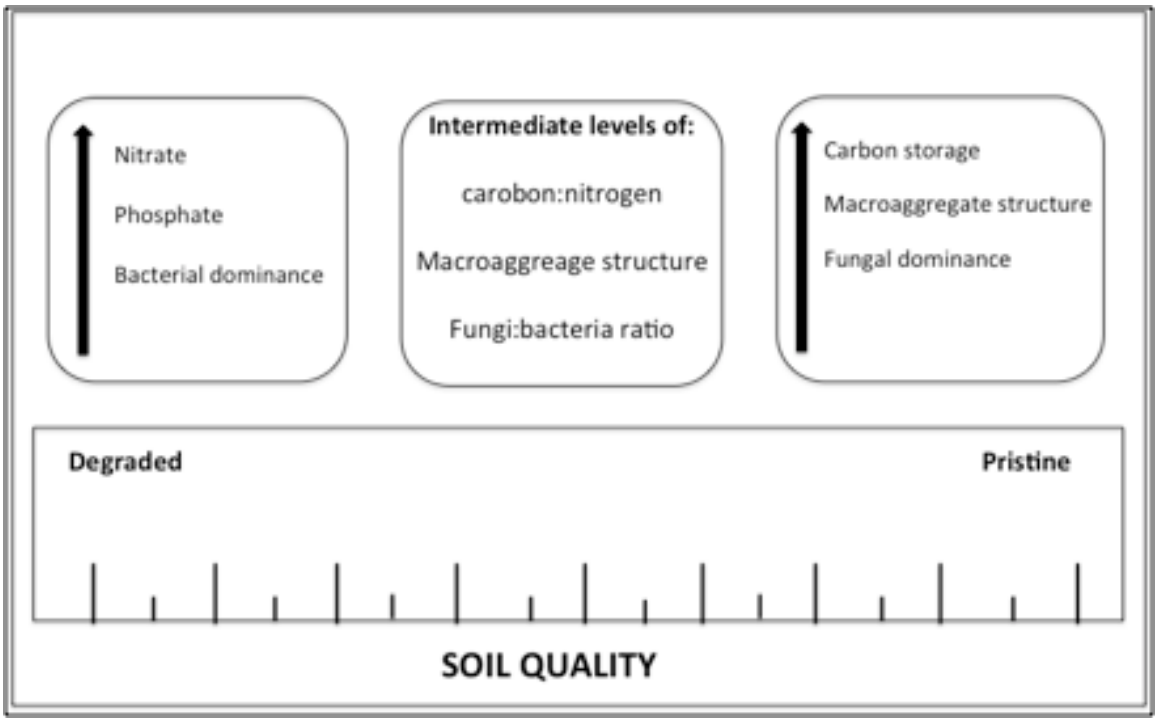

Figure 2. Soil Recovery Model. Proposed status of soil properties across a soil quality gradient. Pristine prairies soils will demonstrate relatively high carbon storage, macroaggregate structure, and fungal abundance. Degraded soils will contain relatively high levels of nitrogen, phosphate, and bacterial abundance. Characteristics of intermediate quality soils will fall along the gradient as depicted above.

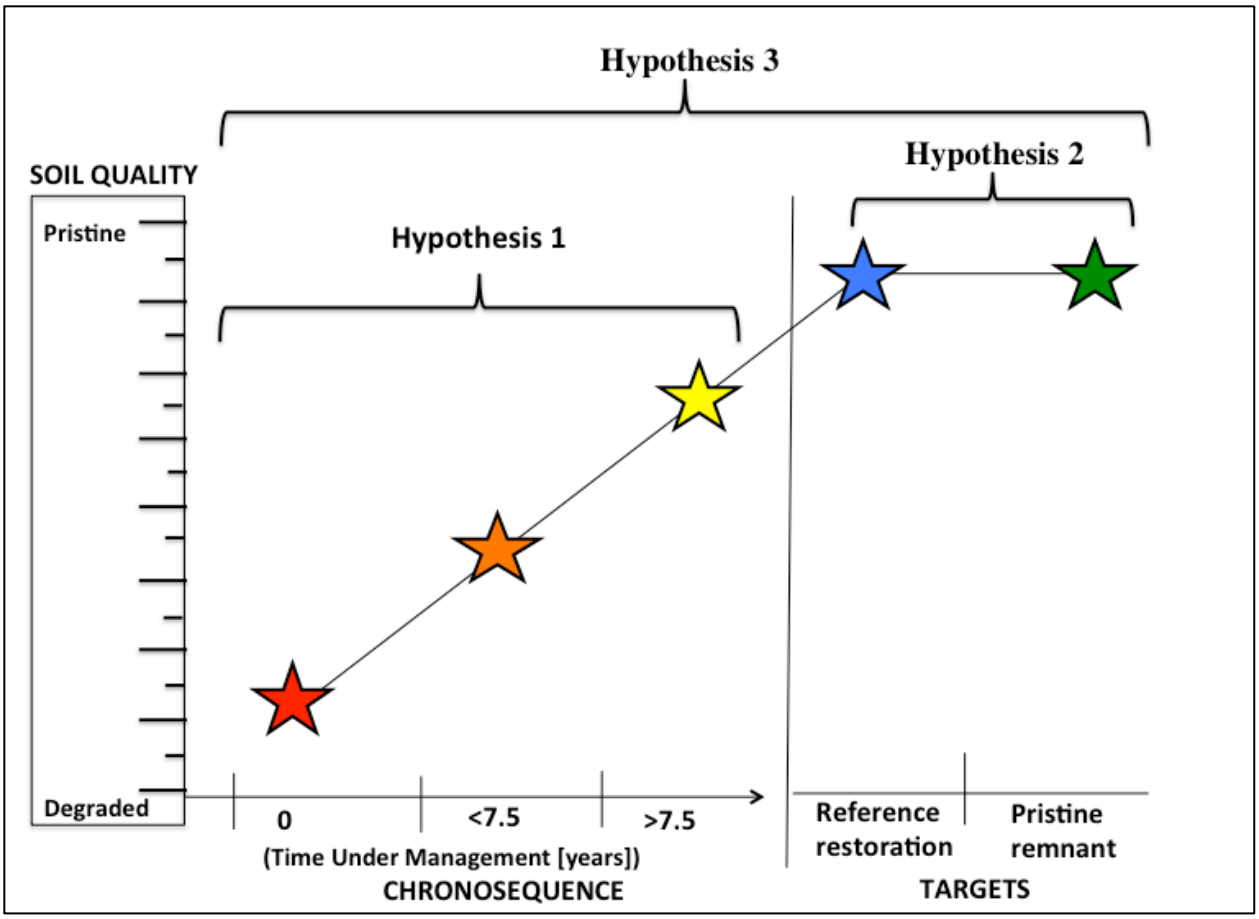

Figure 3. Visual Depiction of Hypotheses. Hypotheses include: 1) soil quality increases with duration of land management, 2) belowground properties of pristine remnants will resemble those of reference restorations 3) pristine remnants and reference restorations will demonstrate greater soil quality (aggregate structure, carbon storage, fertility, and abundance of fungi relative to bacteria) than restorations along the management chronosequence.

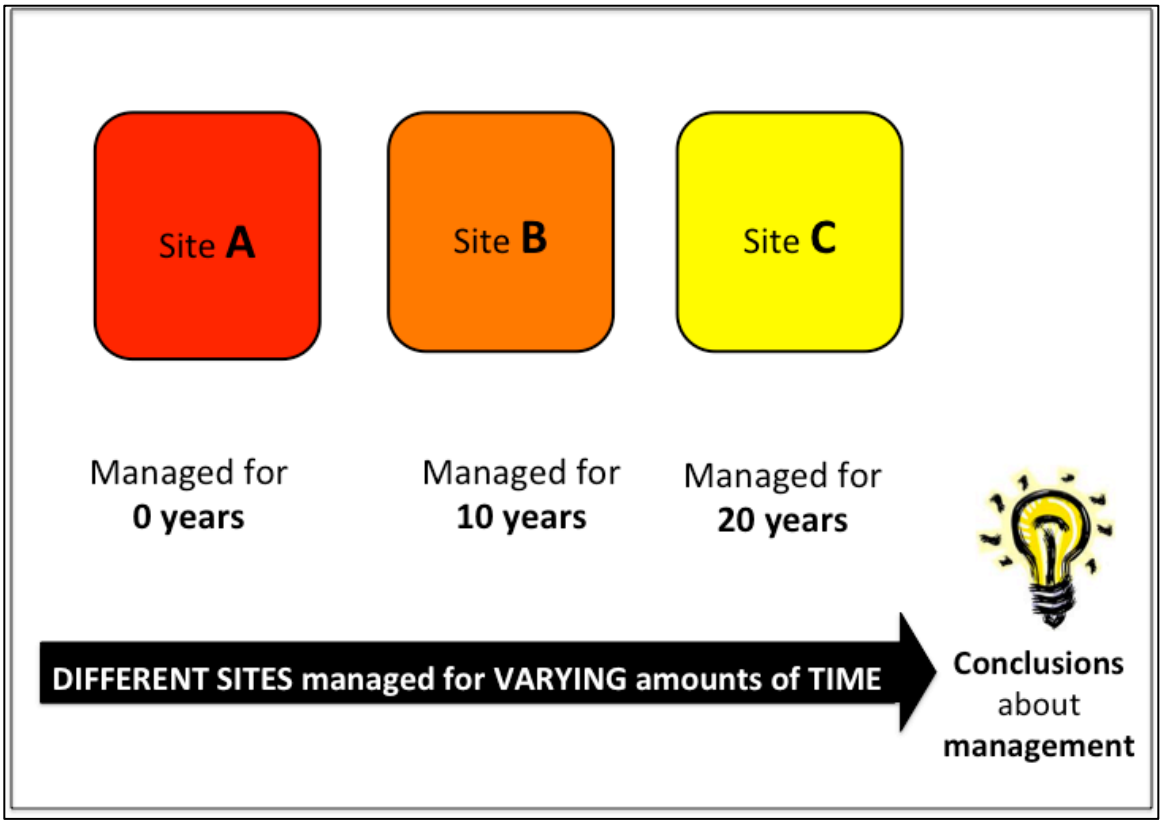

Figure 4. Chronosequence Study Design. Chronosequence studies draw conclusions about ecosystem processes, or the effects of land treatments over time, in a relatively short timeframe. This is possible when multiple sites have been under the same treatment for varying amounts of time, and are similar in most other respects.

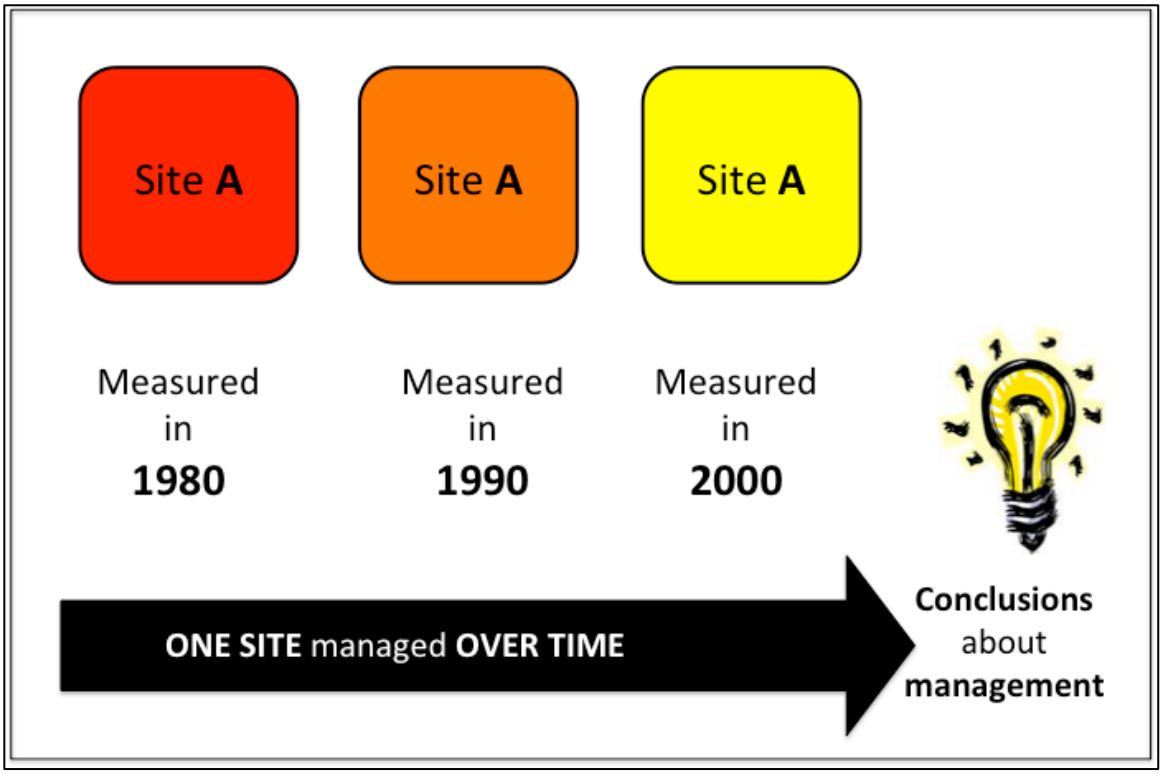

Figure 5. Long Term Study Design. Long-term ecological studies make observations of a single site over an extended time period in order to draw conclusions about ecosystem processes or long-term land treatments.

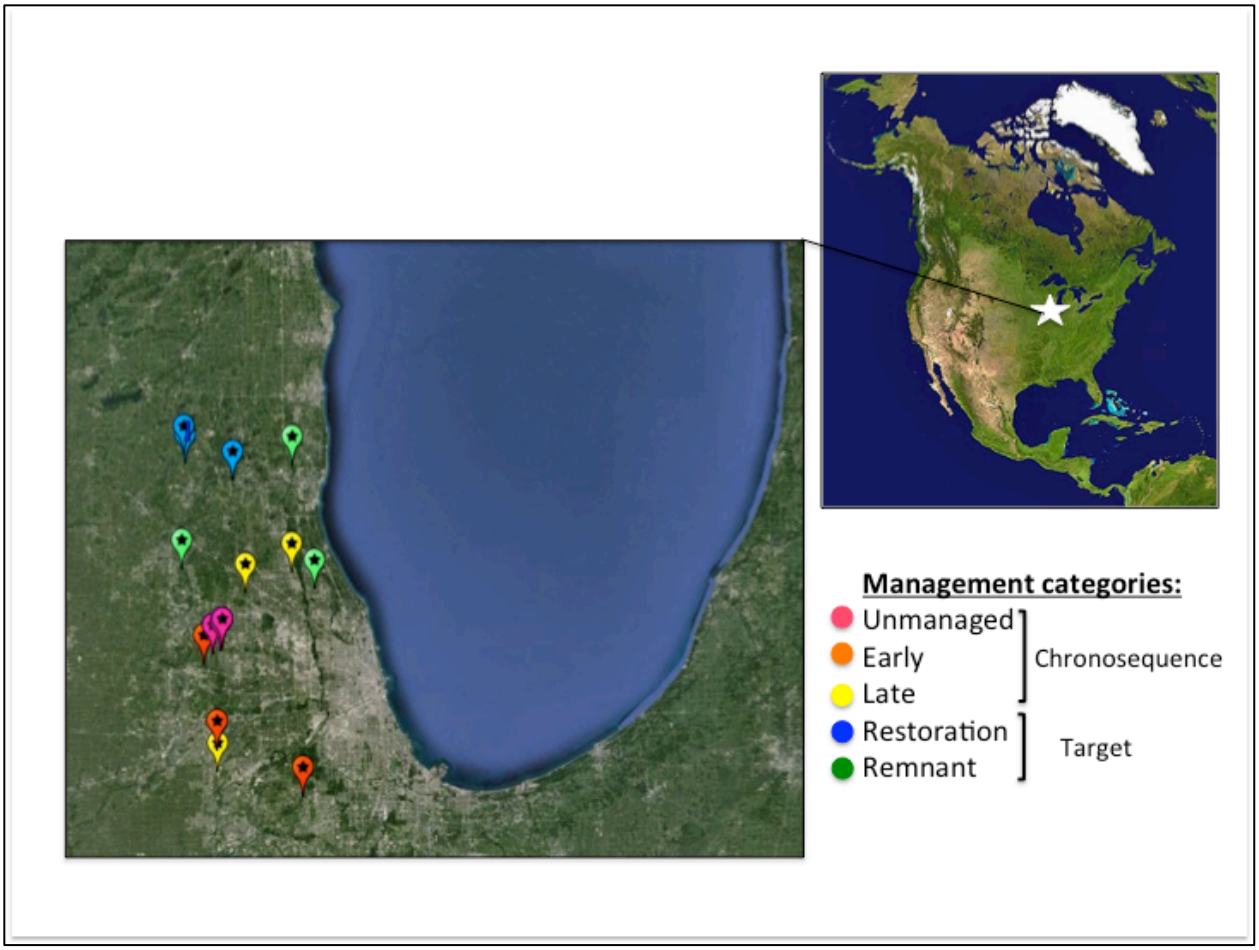

Figure 6. Study Sites. Fifteen study sites were chosen from four counties in the Chicago region (Cook, Lake, McHenry, DuPage). Sites color coded by management category: Red = Unmanaged (R0); Orange = Early-stage management (R1); Yellow = Late-stage management (R2); Blue = Reference Restoration (R3); Green = Pristine Remnant (P3).

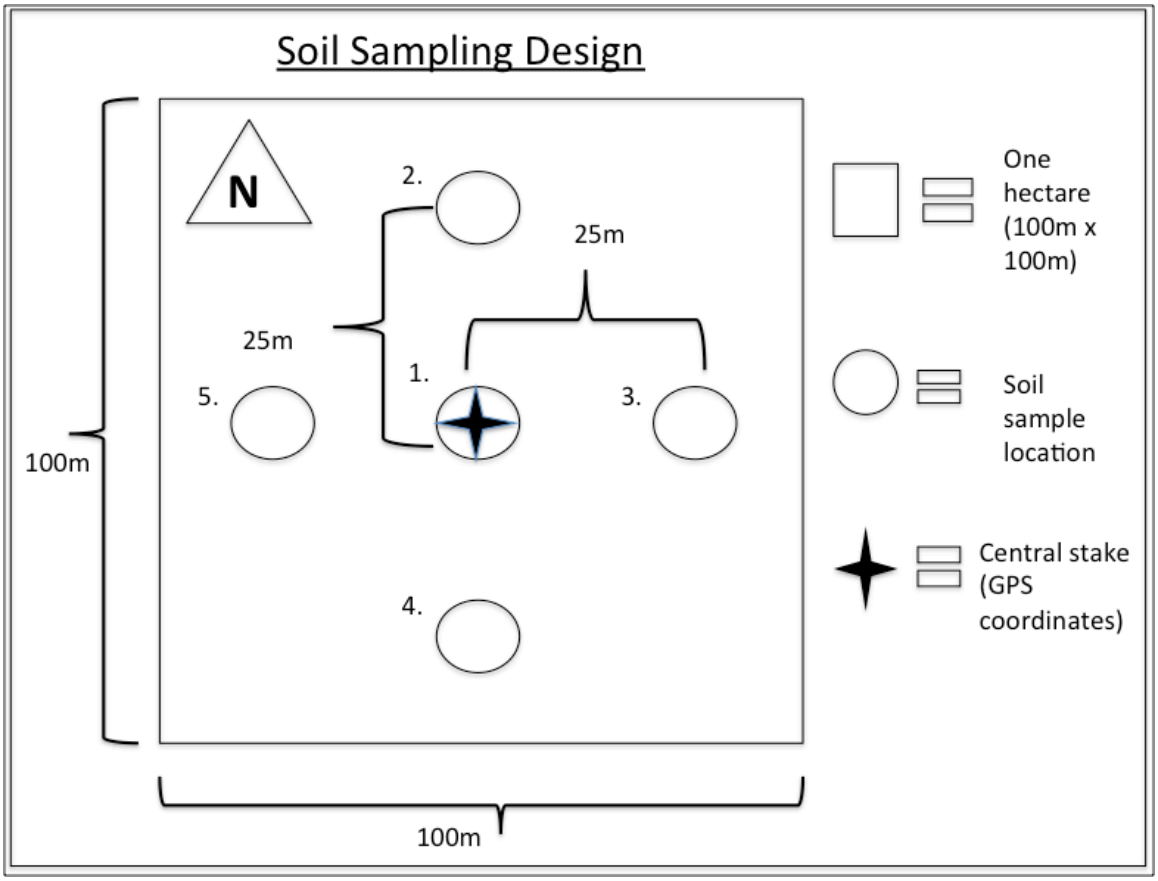

Figure 7. Soil Sampling Design. Five soil samples were collected, at each site, centered around GPS coordinates previously established by the CWLMRP. *This doesn't show up in my version.

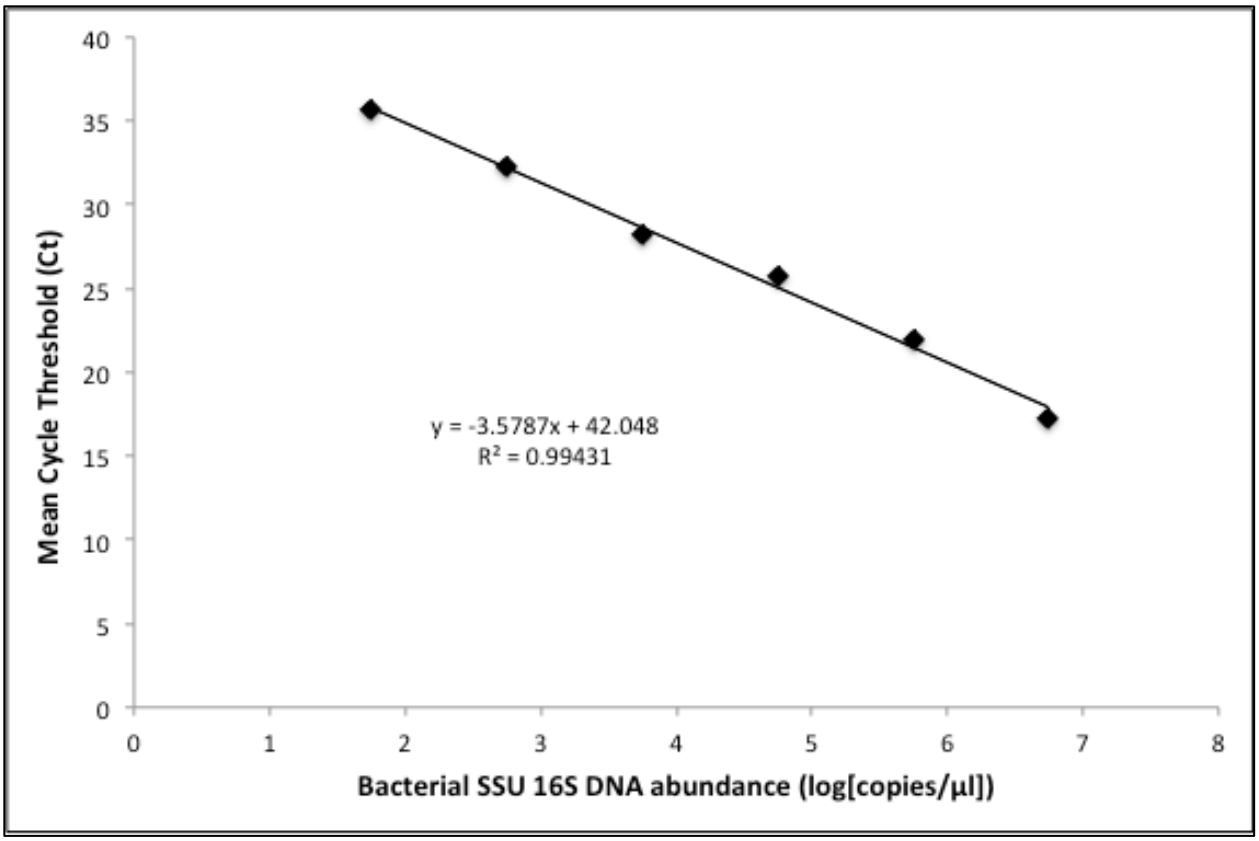

Figure 8a. Standard curve of bacterial SSU 16S rRNA qPCR analysis, replicate 1. Standard curve was derived from PCR products generated by near-full gene amplification of SSU rRNA genes using the general bacterial primer set 27 F and 1492R.

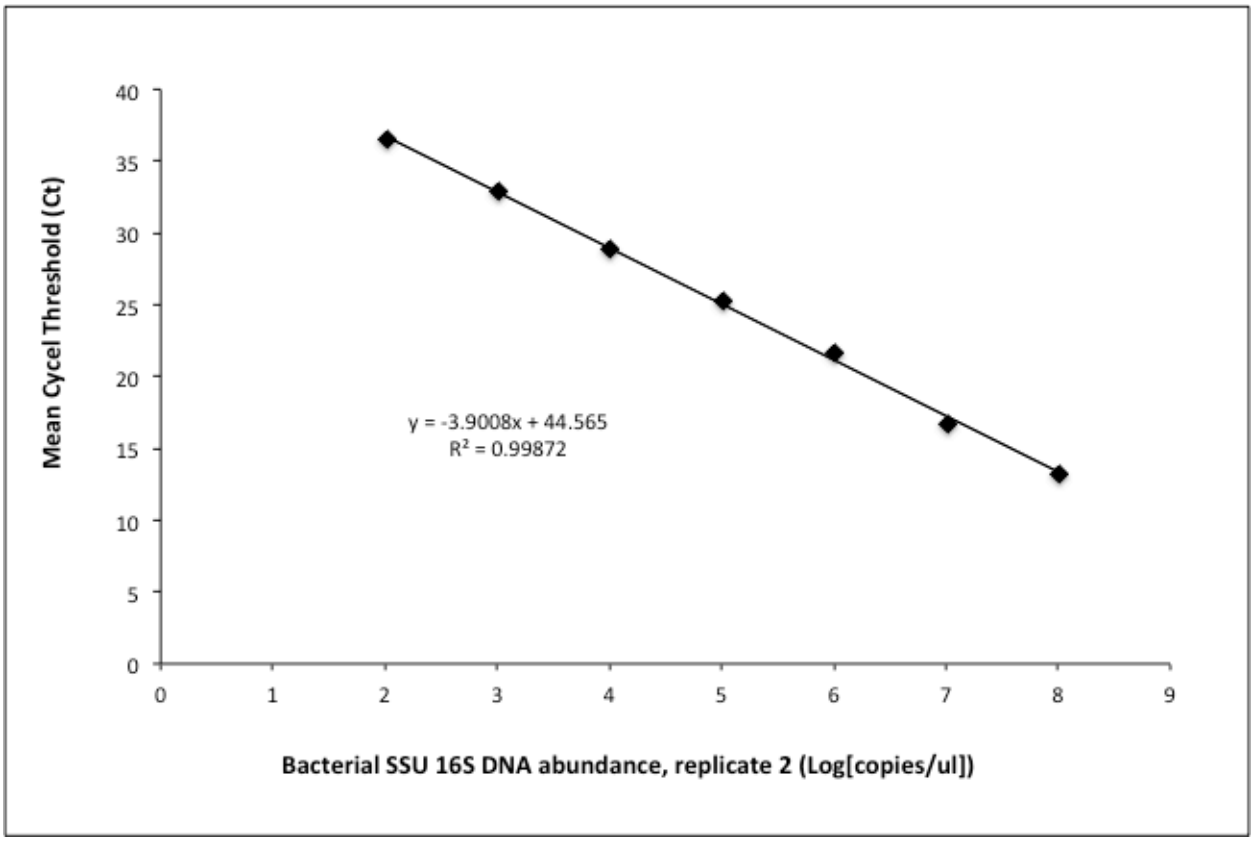

Figure 8b. Standard curve of bacterial SSU 16S rRNA qPCR analysis, replicate 2. Standard curve was derived from PCR products generated by near-full gene amplification of SSU rRNA genes using the general bacterial primer set 27 F and 1492R.

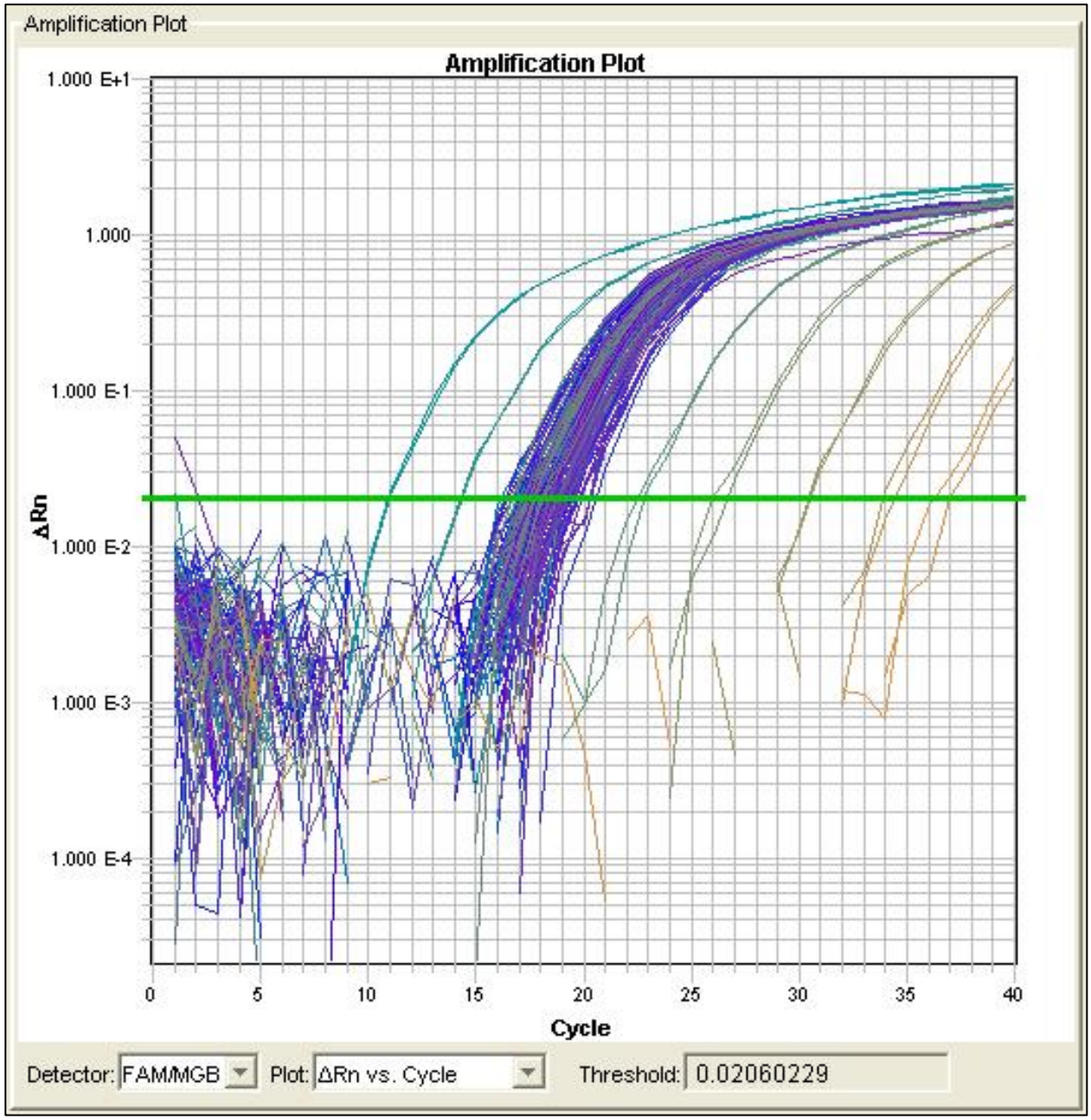

Figure 9. QPCR amplification curve for bacteiral SSU 16S analysis.

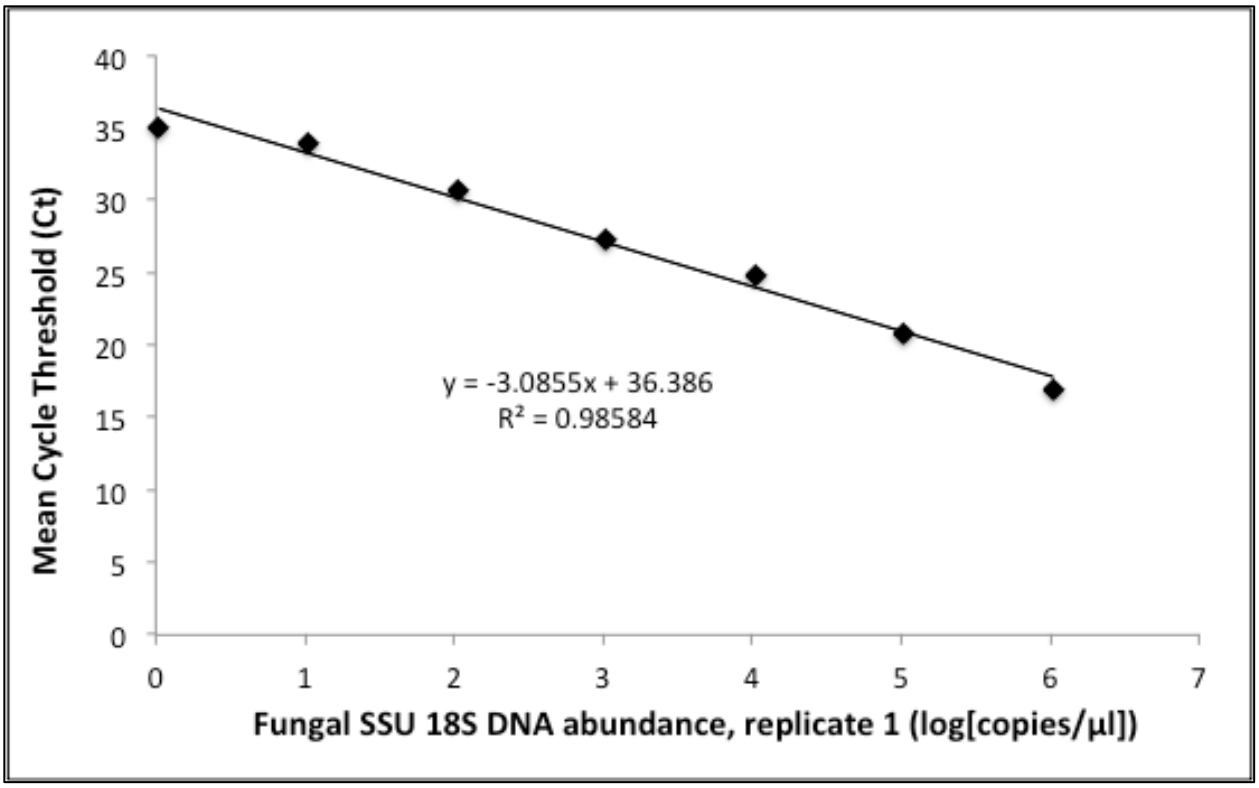

Figure10a. Standard curve of fungal SSU 18S rRNA qPCR analysis, replicate 1. Standard curve was derived from qPCR amplification of general eukaryotic primer product (generated from primer set EukA/B) using general fungal 18S rRNA primer set FR1/FF390 and SYBR Green.

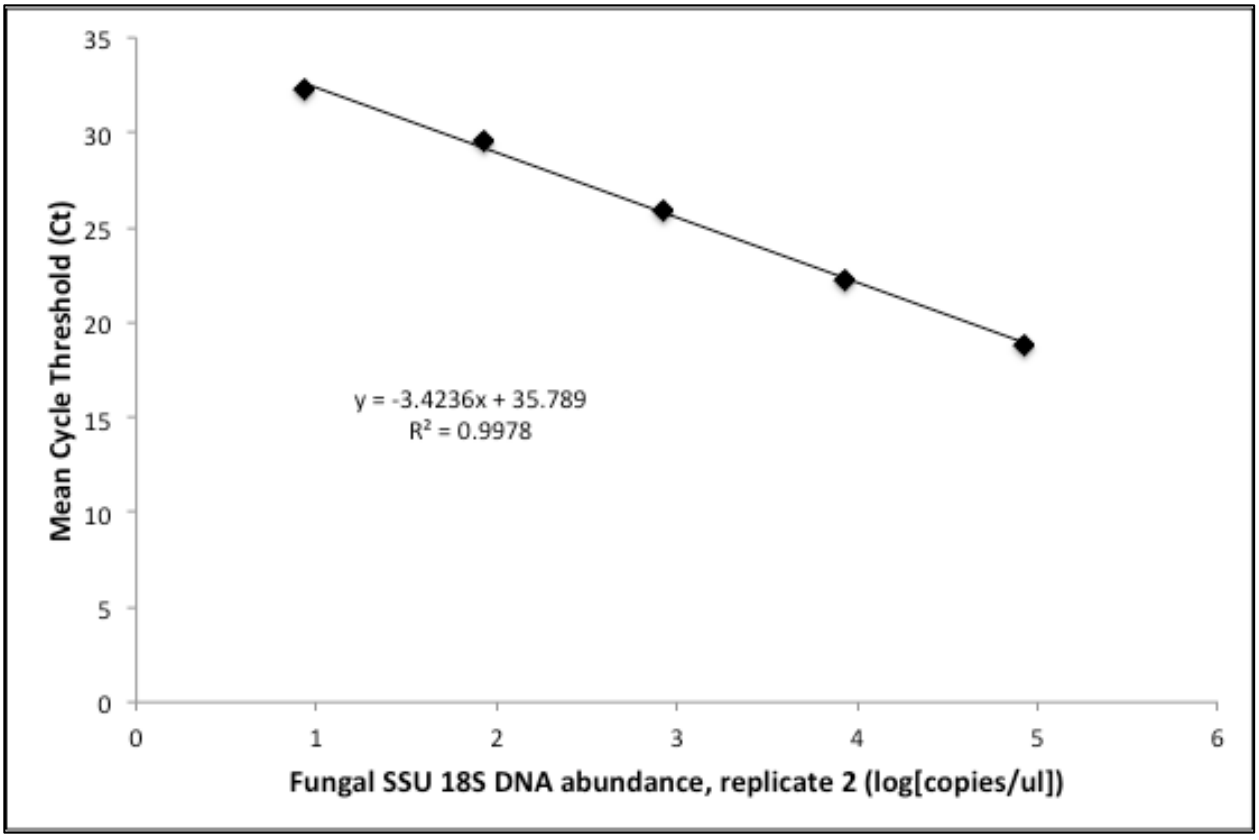

Figure 10b. Standard curve of fungal SSU 18S rRNA qPCR analysis, replicate 2. Standard curve was derived from qPCR amplification of general eukaryotic primer product (generated from primer set EukA/B) using general fungal 18S rRNA primer set FR1/FF390 and SYBR Green.

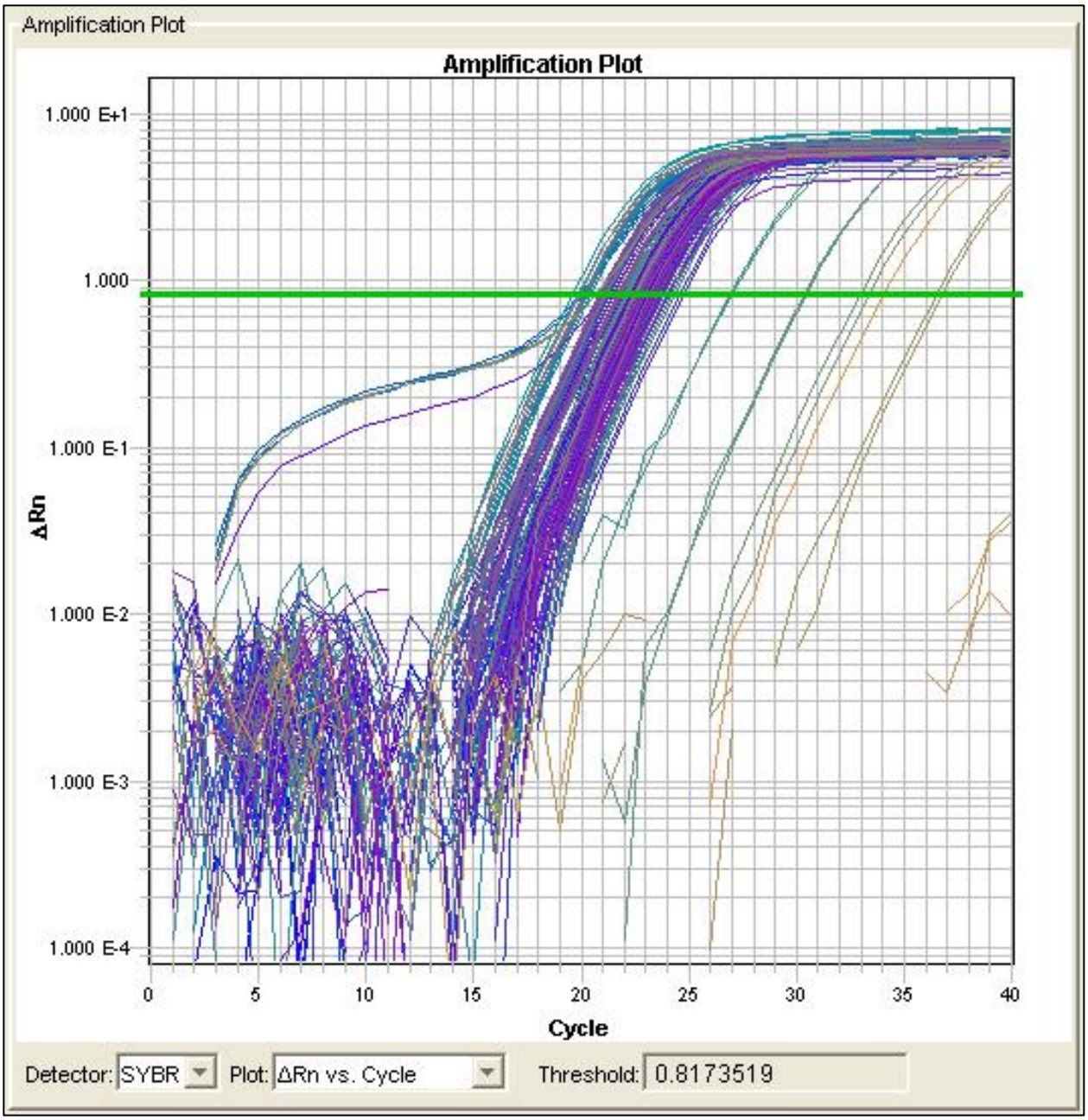

Figure 11. QPCR amplification curve for fungal SSU 18S analysis.

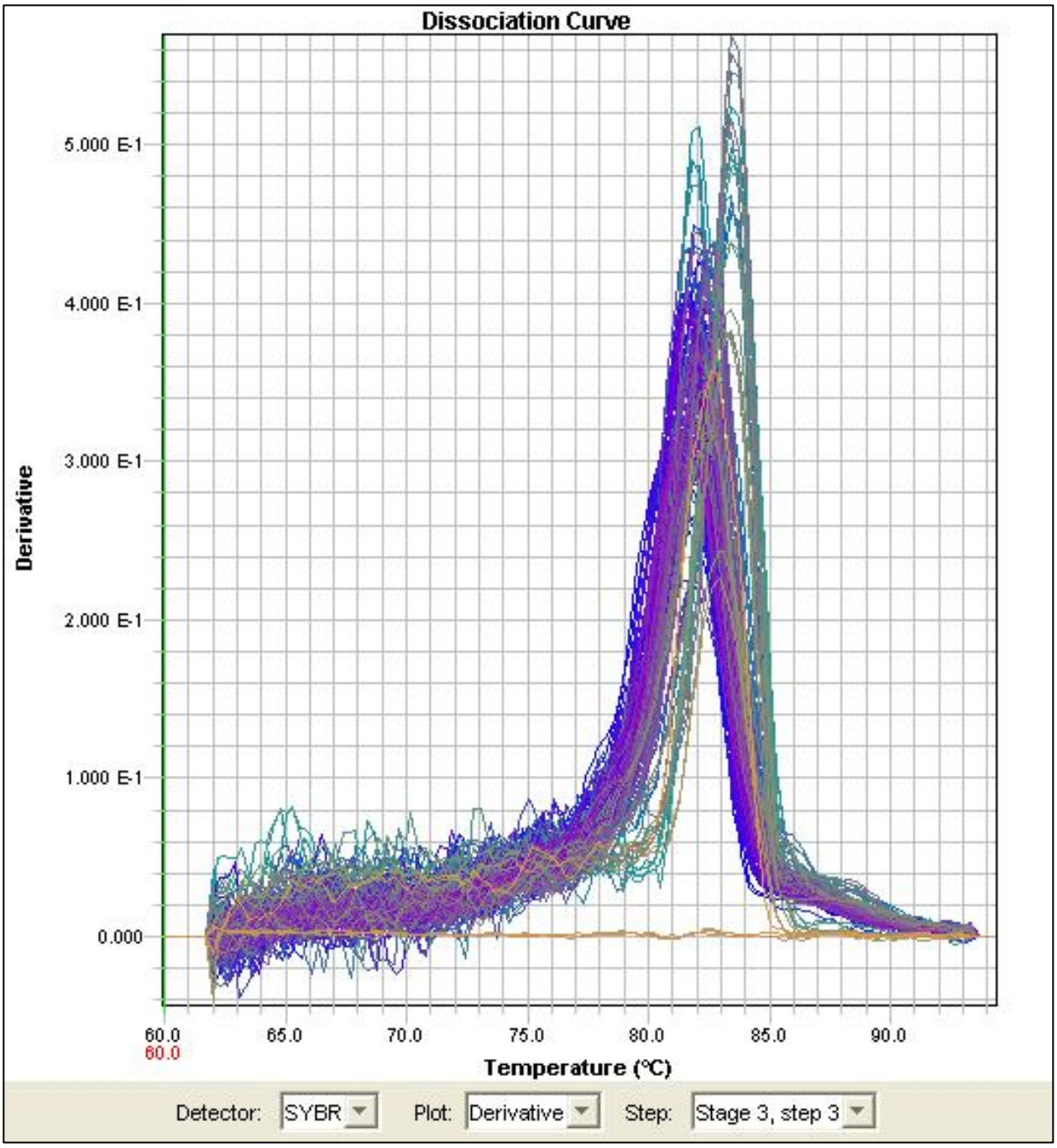

Figure 12. QPCR melt peak for fungal SSU 18S analysis.

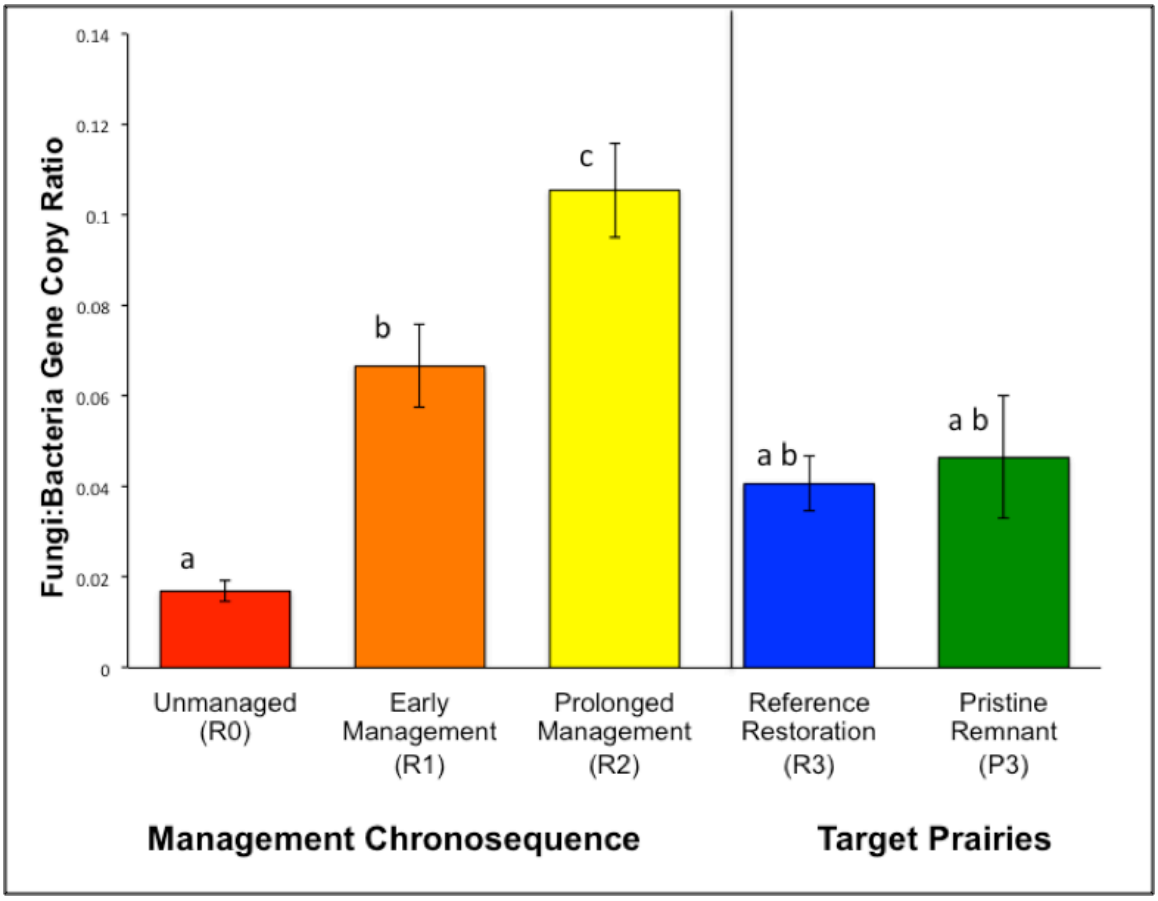

Figure 13. Fungal:bacterial Gene Copy Ratios in Managed and Target Prairies. Quantitative microbial community analysis using qPCR to estimate the ratio of fungal: bacterial copy numbers under different management regimes. Vertical bars indicate standard error of the mean. Bars with the same letter do not differ significantly at $P < 0.05$ (Tukey's HSD).

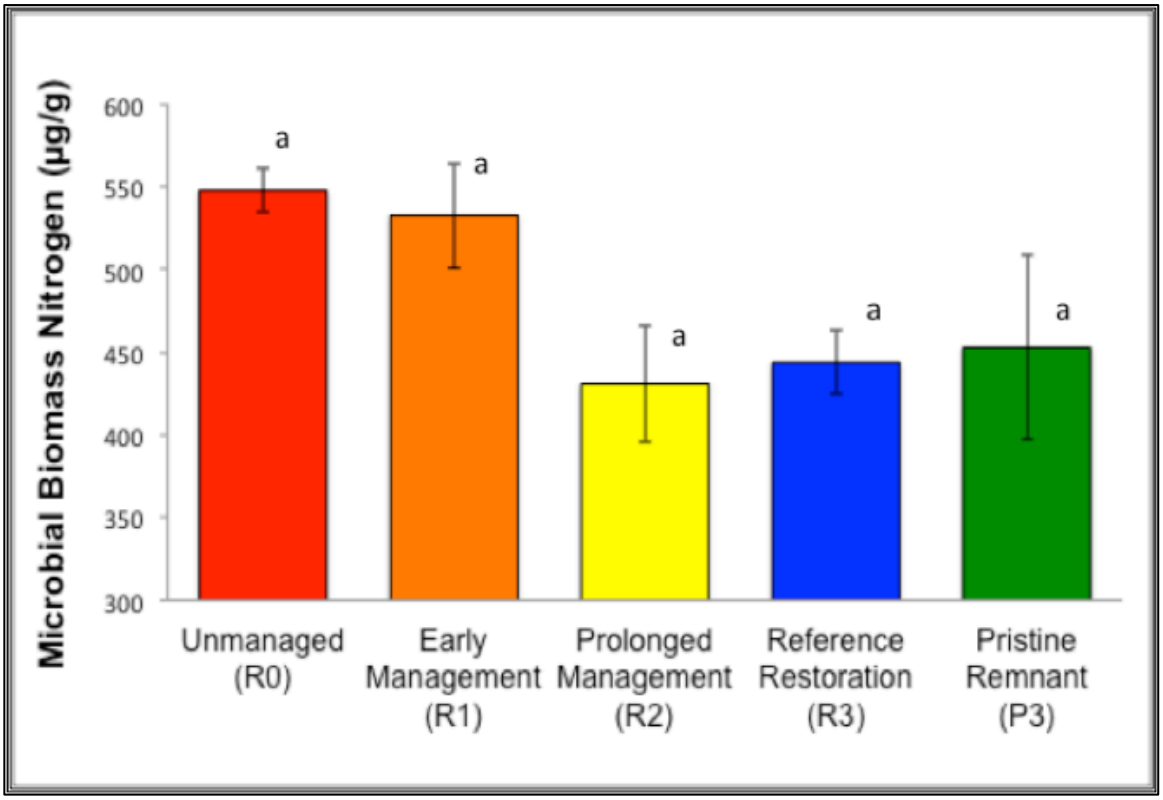

Figure 14. Microbial biomass-N in managed and target prairies. Microbial biomass nitrogen along a management sequence in reference and remnant prairies. Vertical bars indicate the standard errors of the mean. Bars with the same letter do not differ significantly at $P<0.05$ (Tukey's HSD).

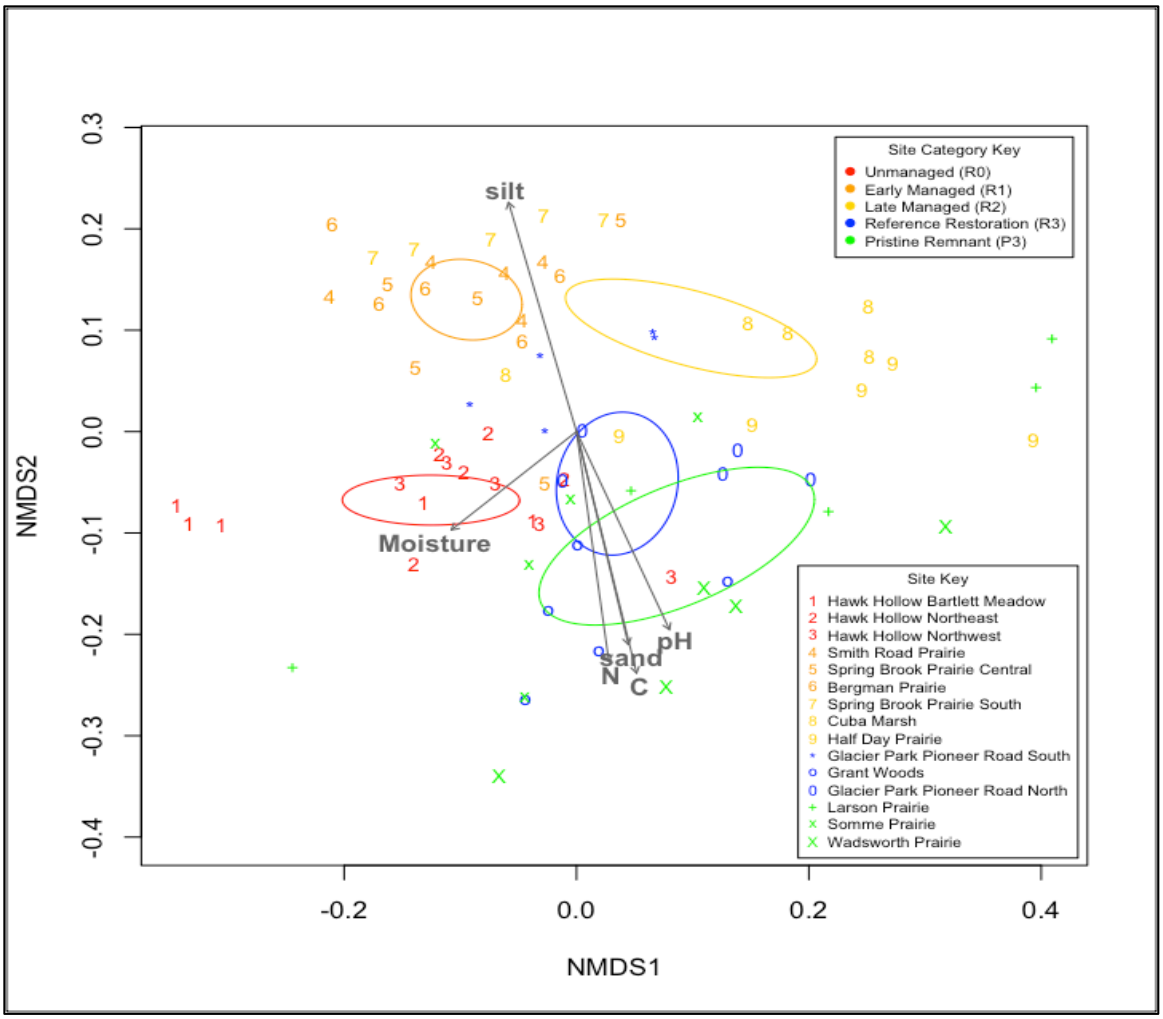

Figure 15. Ordination of biological soil properties overlaid with abiotic drivers. The arrangement of all study sites in ordination space, using non-metric multidimensional scaling (NMDS). Individual soil samples are numbered by site, and color-coded by site category. Ellipses are similarly color-coded by site category, and depict a central tendency for each management category, deliniated by 1 standard error from the mean. The environmental vectors for % C, % N, pH, % silt, % sand, and gravimetric moisture were derived from a post-hoc PERMANOVA analysis, and represent significant ($p < 0.01$) physical and chemical factors influencing the assembly of soil samples. The direction of the vectors indicates the orientation of that factor's trajectory in which the samples assembled. Vector lengths are relative to the correlation coefficients for each variable.

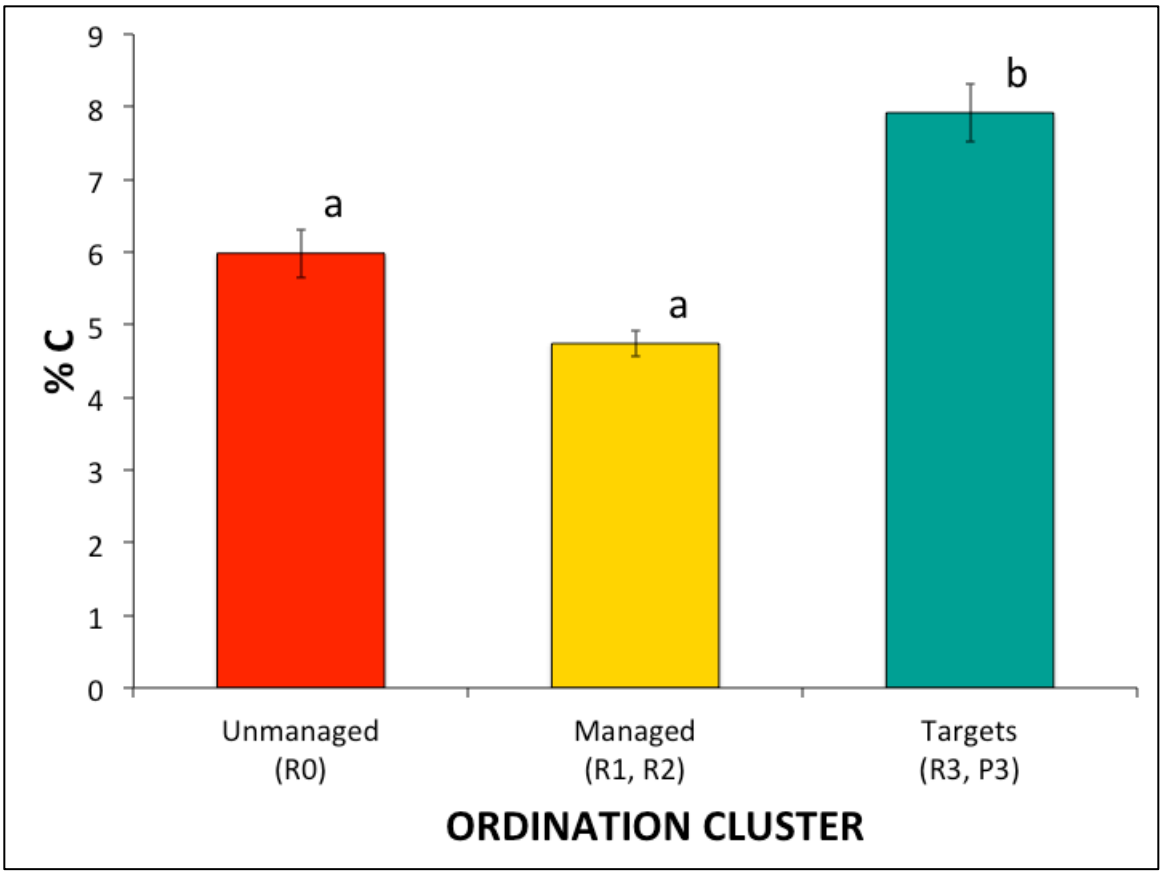

Figure 16. Mean % C of sites clustered together in NMDS ordination space. Three site clusters emerged from the NMDS ordination arrangement, including: 1) unmanaged old fields (R0), 2) managed prairie restorations (R1 and R2), and C) target prairies (R3 and P3). Bars with the same letter are not significantly different ($p<0.05$) based on Tukey's HSD mean comparison tests.

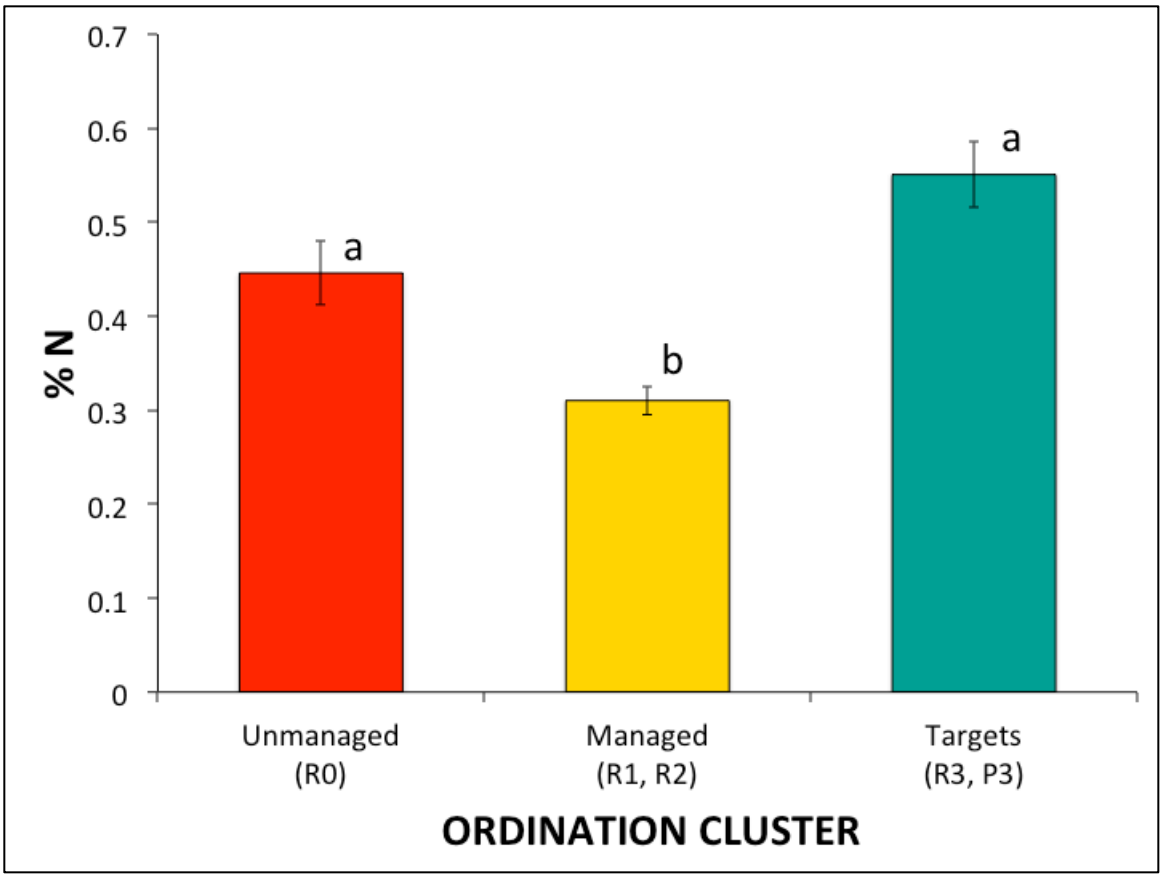

Figure 17. Mean % N of sites clustered together in NMDS ordination space. Three site clusters emerged from the NMDS ordination arrangement, including: 1) unmanaged old fields (R0), 2) managed prairie restorations (R1 and R2), and C) target prairies (R3 and P3). Bars with the same letter are not significantly different ($p<0.05$) based on Tukey's HSD mean comparison tests.

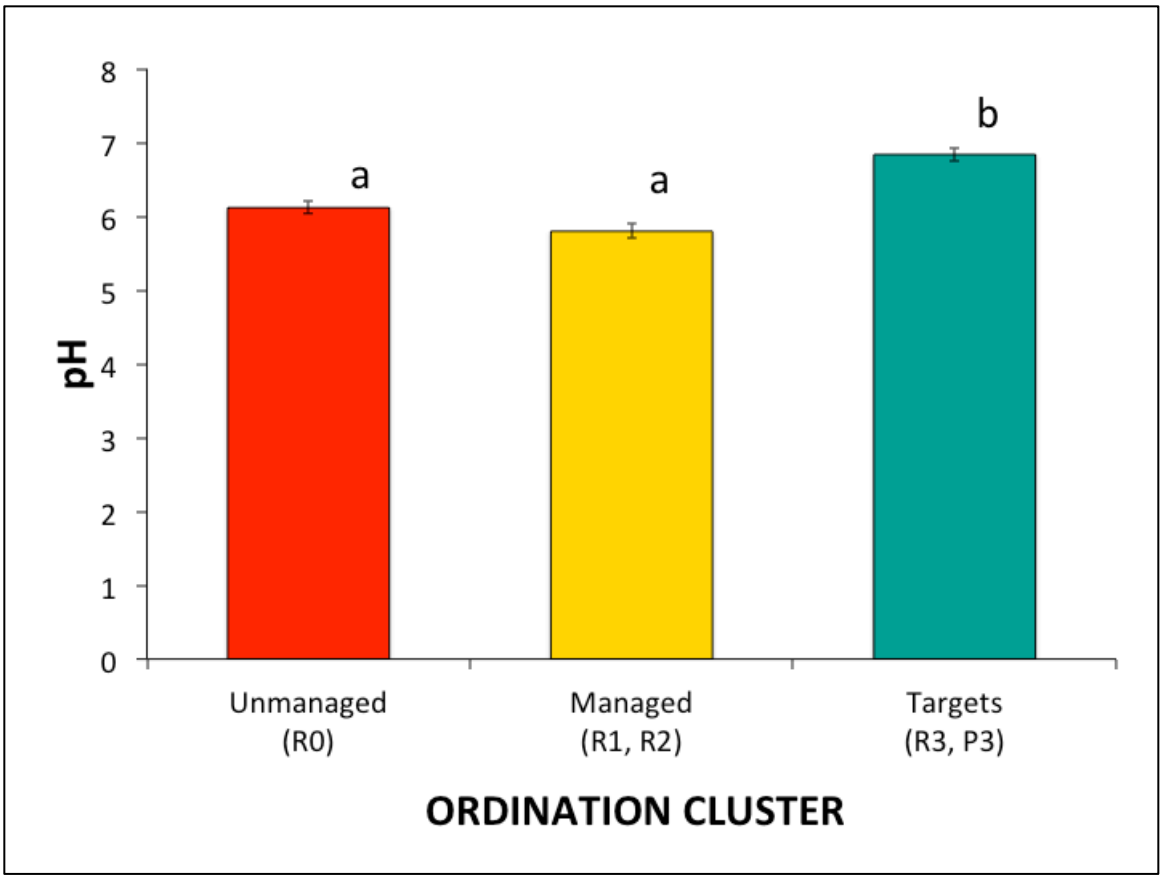

Figure 18. Mean pH of sites clustered together in NMDS ordination space. Three site clusters emerged from the NMDS ordination arrangement, including: 1) unmanaged old fields (R0), 2) managed prairie restorations (R1 and R2), and C) target prairies (R3 and P3). Bars with the same letter are not significantly different ($p<0.05$) based on Tukey's HSD mean comparison tests.

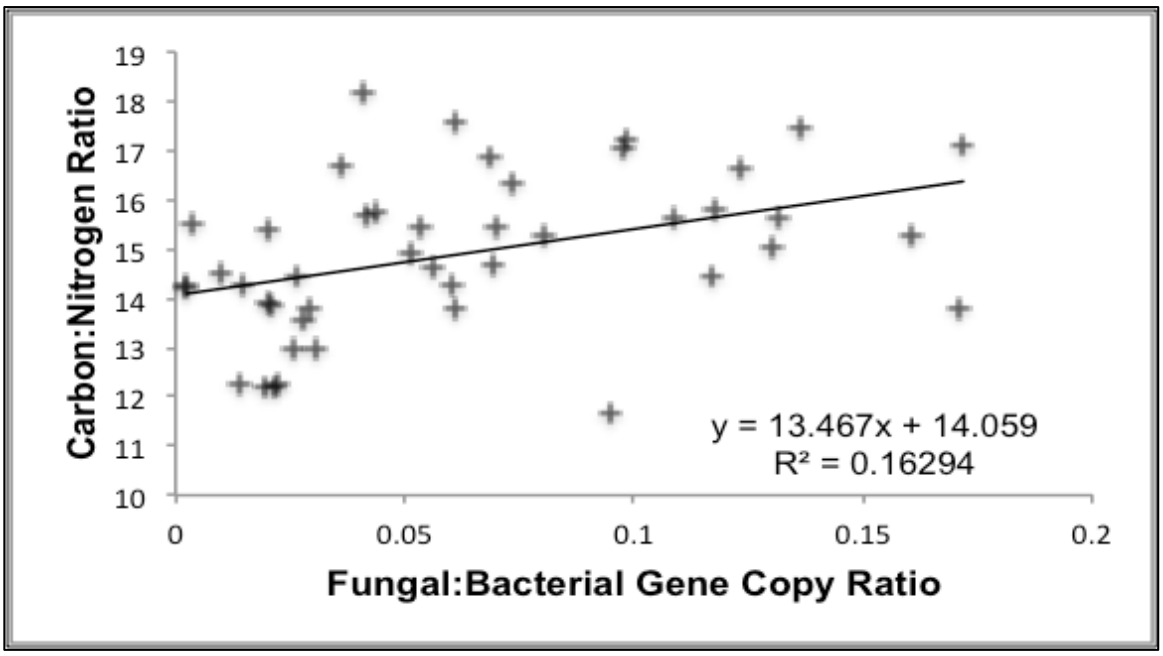

Figure 19. Change in F: B gene copy ratio with increasing soil C: N ratios in prairie restorations across the management chronosequence (R0, R1, R2). Linear regression plot of C:N ratios fit by fungal:bacterial gene copy ratios in prairie restorations across the management chronosequence (R0, R1, R2); R^2=0.162944, p=0.0060, y=13.467x+14.059.

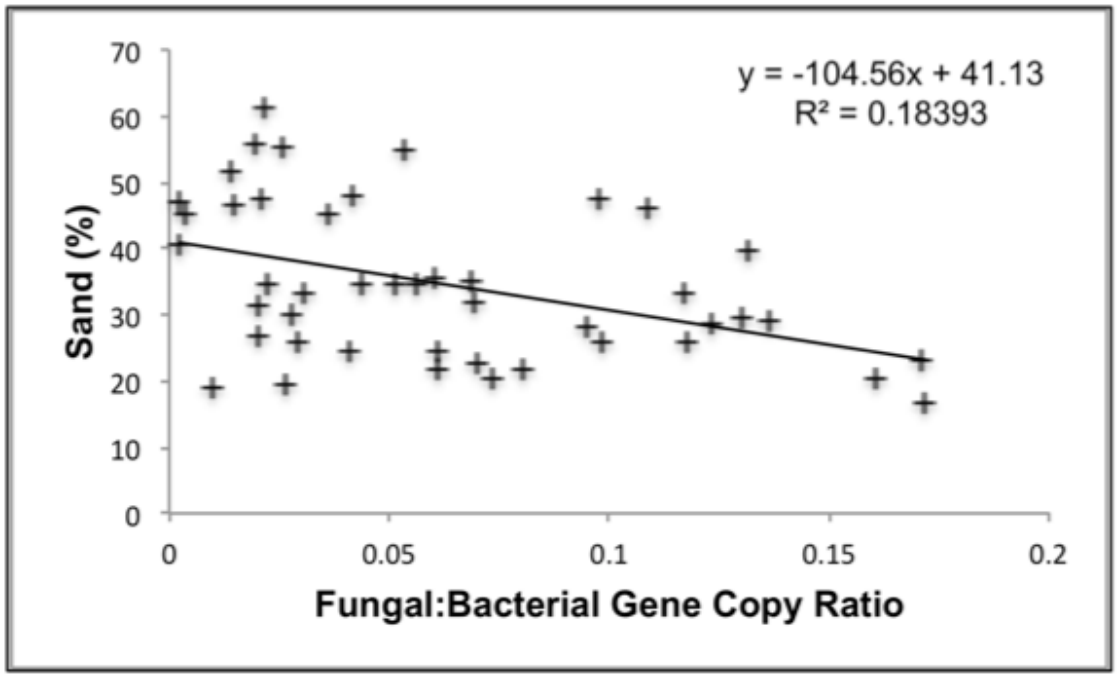

Figure 20. Change in F: B gene copy ratio with increasing % sand in prairie restorations across the management chronosequence (R0, R1, R2). Linear Regression Plot of Sand as predicted by Fungal:Bacterial Gene Copy Ratio in Prairie Restorations Along the Management Chronosequence (R0, R1, R2); R2=0.183928, p=0.0033, y=-104.56x+41.13.

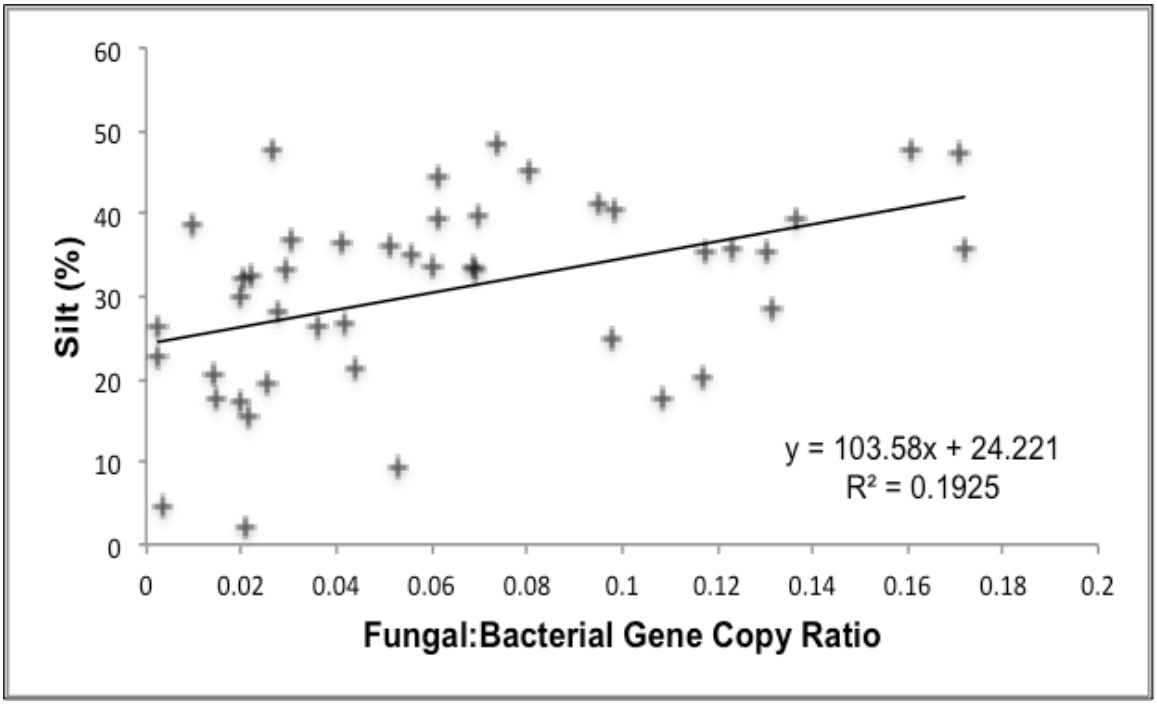

Figure 21. Change in F: B gene copy ratio with increasing % silt in prairie restorations across the management chronosequence (R0, R1, R2). Linear Regression Plot of Silt as predicted by Fungal:Bacterial Gene Copy Ratio in Prairie Restorations Along the Management Chronosequence (R0, R1, R2); R^2=0.192499, p=0.0026, y=103.58x+24.221.

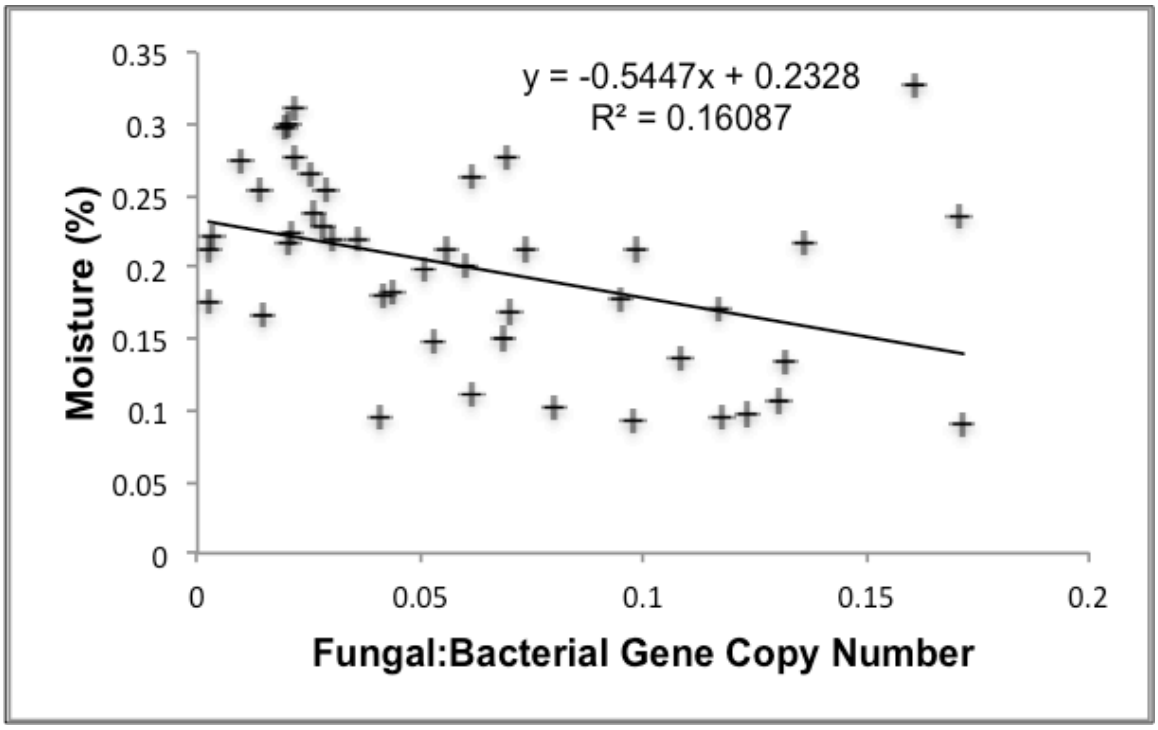

Figure 22. Change in F:B with increasing soil gravimetric moisture content in prairie restorations across the management chronosequence (R0, R1, R2). Linear regression plot of moisture as predicted by fungal:bacterial gene copy ratio in prairie restorations along the management chronosequence (R0, R1, R2); R^2=0.16087, p=0.0063, y=-0.5447x+0.2328.

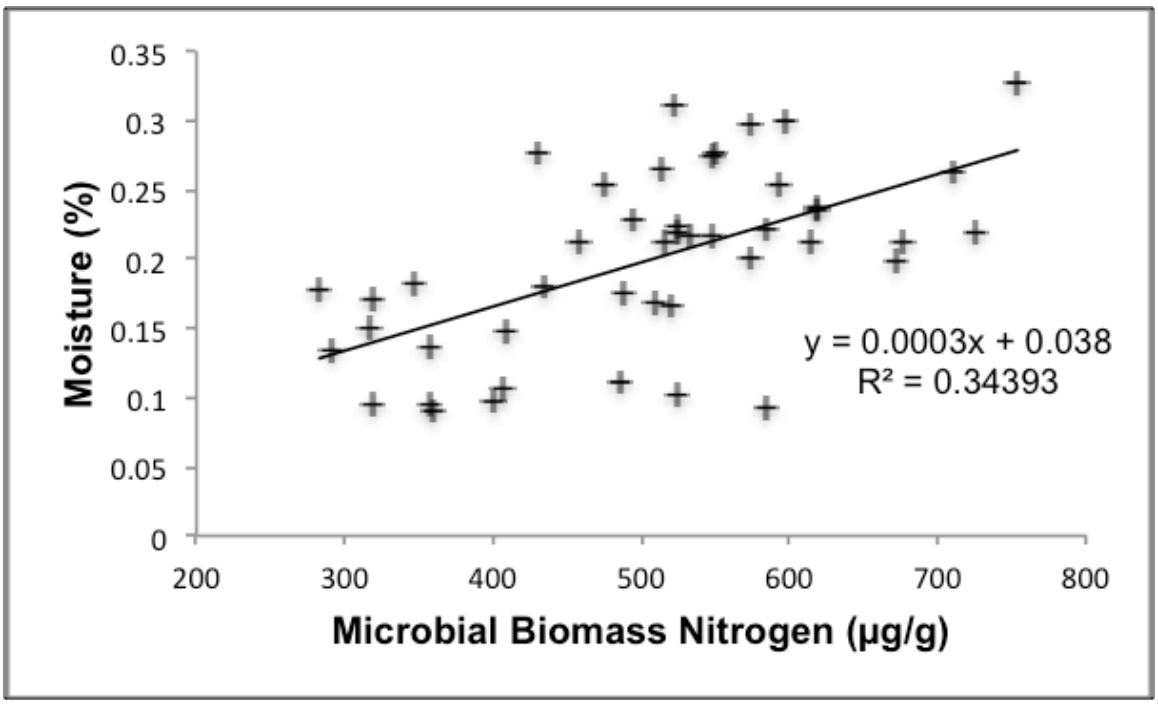

Figure 23. Change in soil microbial biomass N with increasing soil gravimetric moisture content in prairie restorations across the management chronosequence (R0, R1, R2). Linear Regression Plot of Moisture as predicted by Microbial Biomass Nitrogen levels in Prairie Restorations Along the Management Chronosequence (R0, R1, R2); $R2=0.34393$, $p<0.0001$, $y=0.0003x+0.038$.

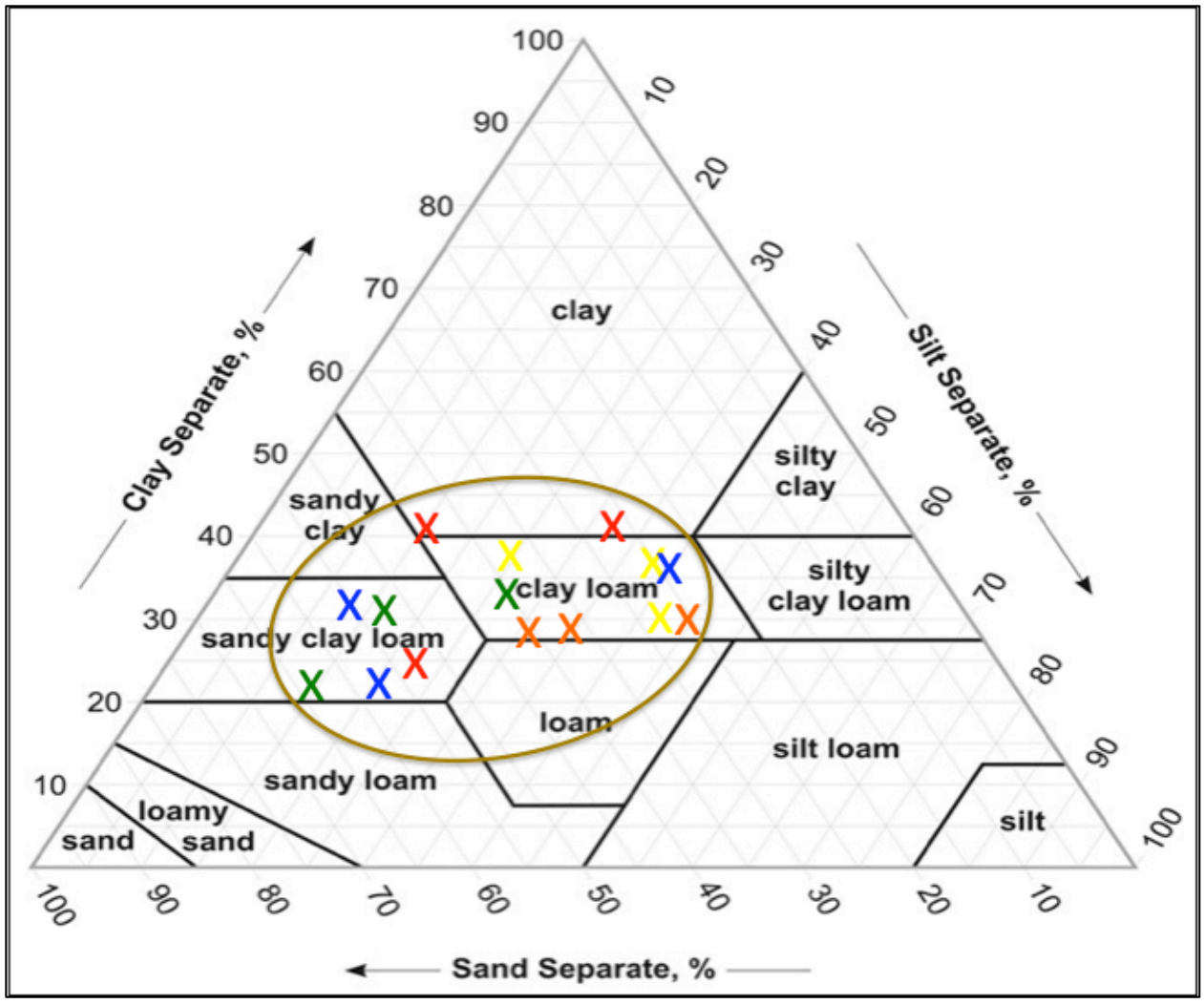

Figure 24. Soil texture triangle overlaid with mean soil texture by site. Data collected in July 2012. Markers (X's) represent mean values of % silt, % sand, and % clay by site (n=15). Color code key: Red = Unmanaged (R0), Orange = Early-stage management (R1), Yellow = Prolonged management (R2), Blue = Reference Restoration (R3), Green = Pristine remnant (P3).

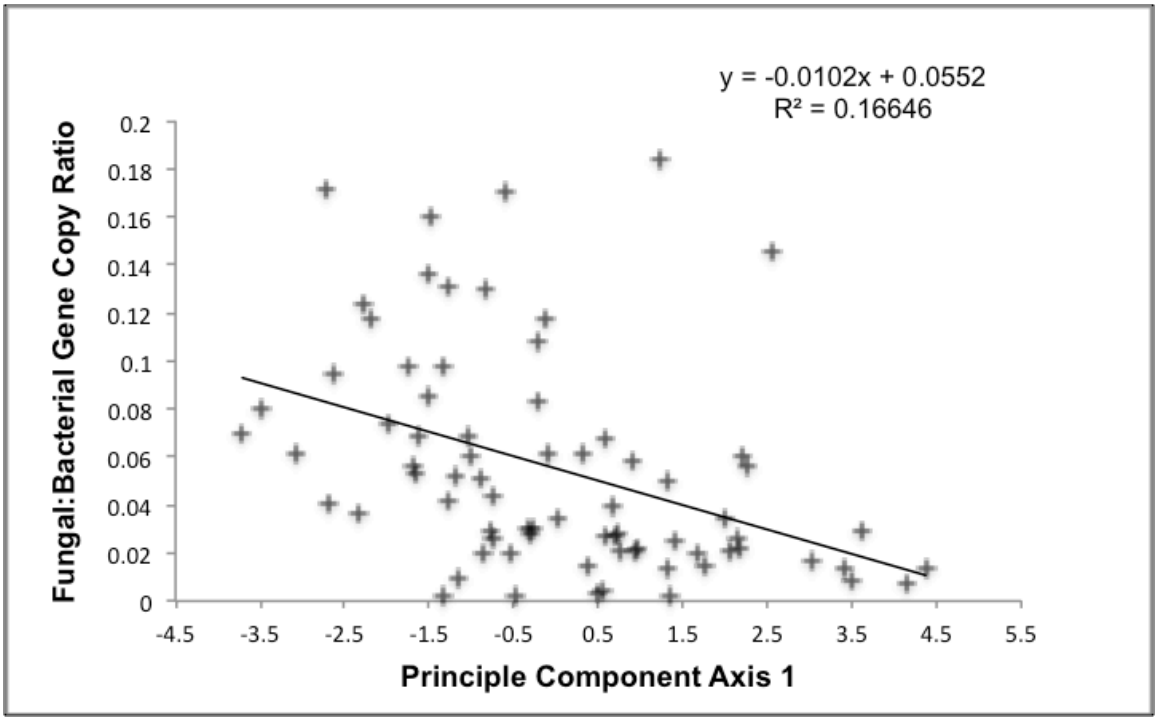

Figure 25. The relationship between F: B gene copy ratio and PCA1 (soil fertility). Linear regression of fungal:bacterial gene copy ratio as predicted by the total principle component analysis axis 1 variation of NMDS-significant physical and chemical soil variables (55.2%); R^2=0.16646, y=-0.0102x+0.0552, p=0.0003).

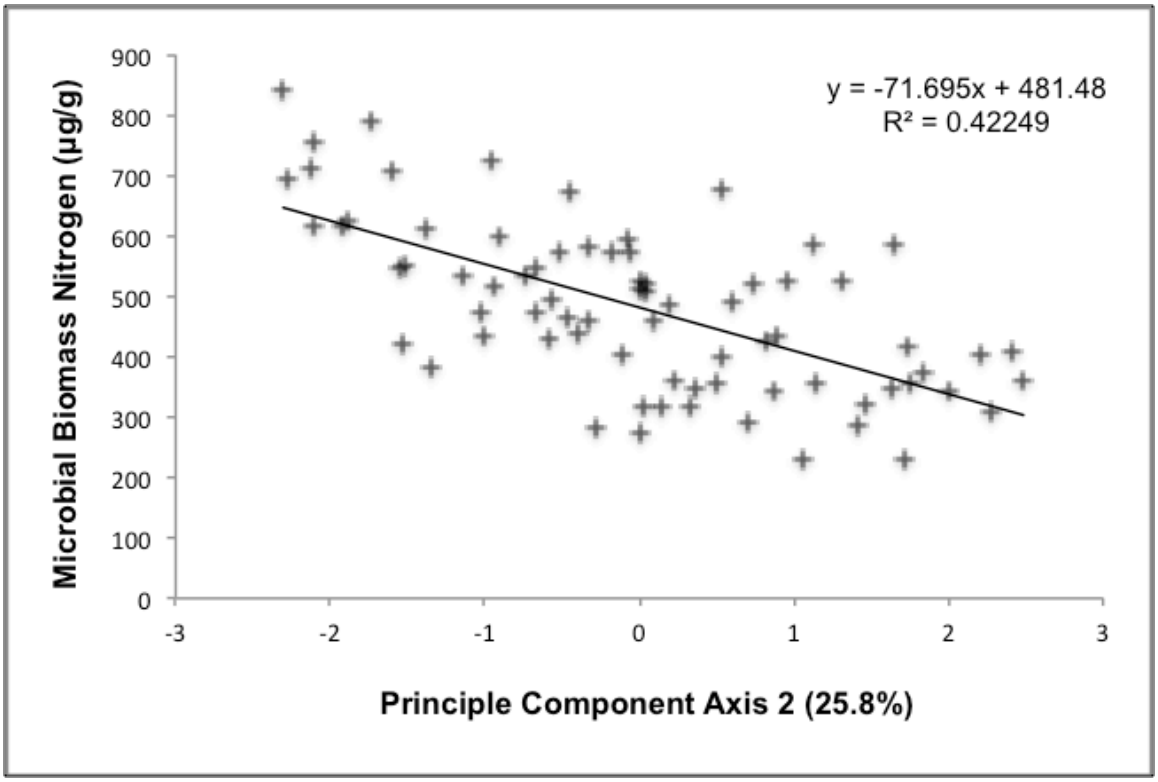

Figure 26. The relationship between F: B gene copy ratio and PCA2 (soil texture, moisture). Linear regression of microbial biomass nitrogen as predicted by the total principle component analysis axis 2 variation of NMDS-significant physical and chemical soil variables (25.8%); R^2=0.42249, y=-71.695x+481.48, p<0.0001.

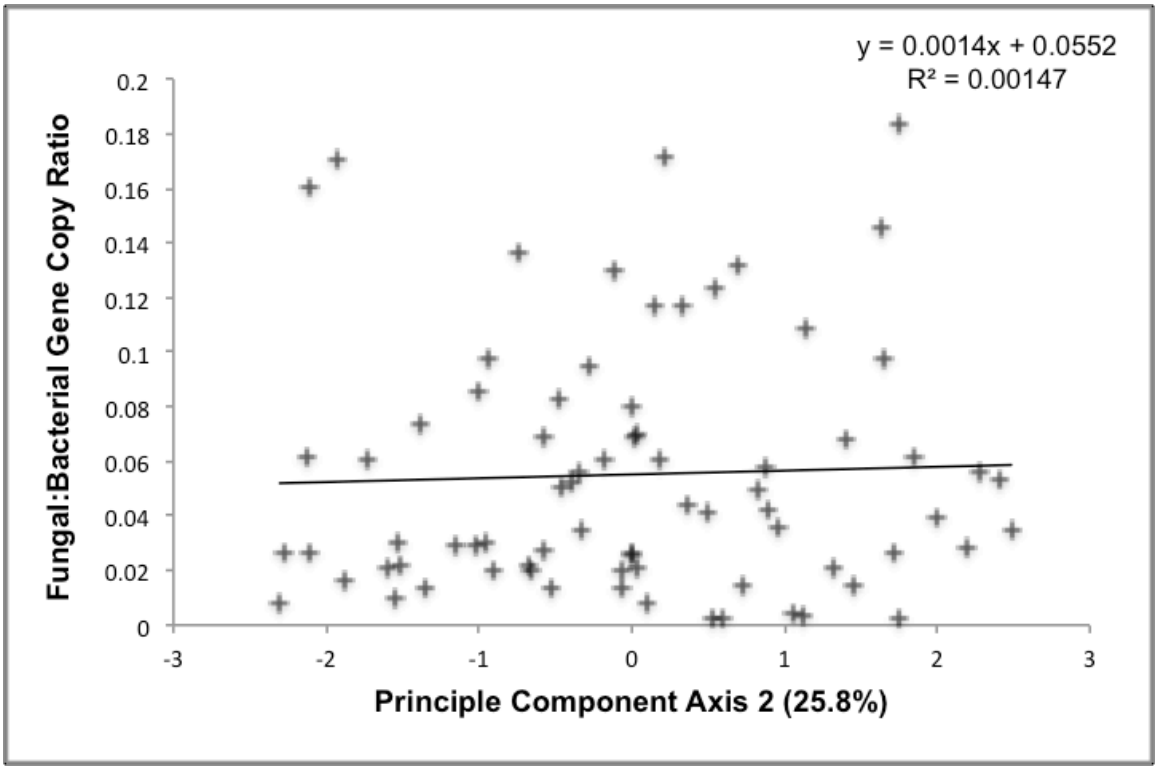

Figure 27. The relationship between F: B gene copy ratio and PCA2 (soil texture, moisture). Linear regression of fungal:bacterial gene copy ratio as predicted by the total principle component analysis axis 2 variation of NMDS-significant physical and chemical soil variables (25.8%); R^2=0.00147, y=-0.0014x+0.0552, p=0.7442.

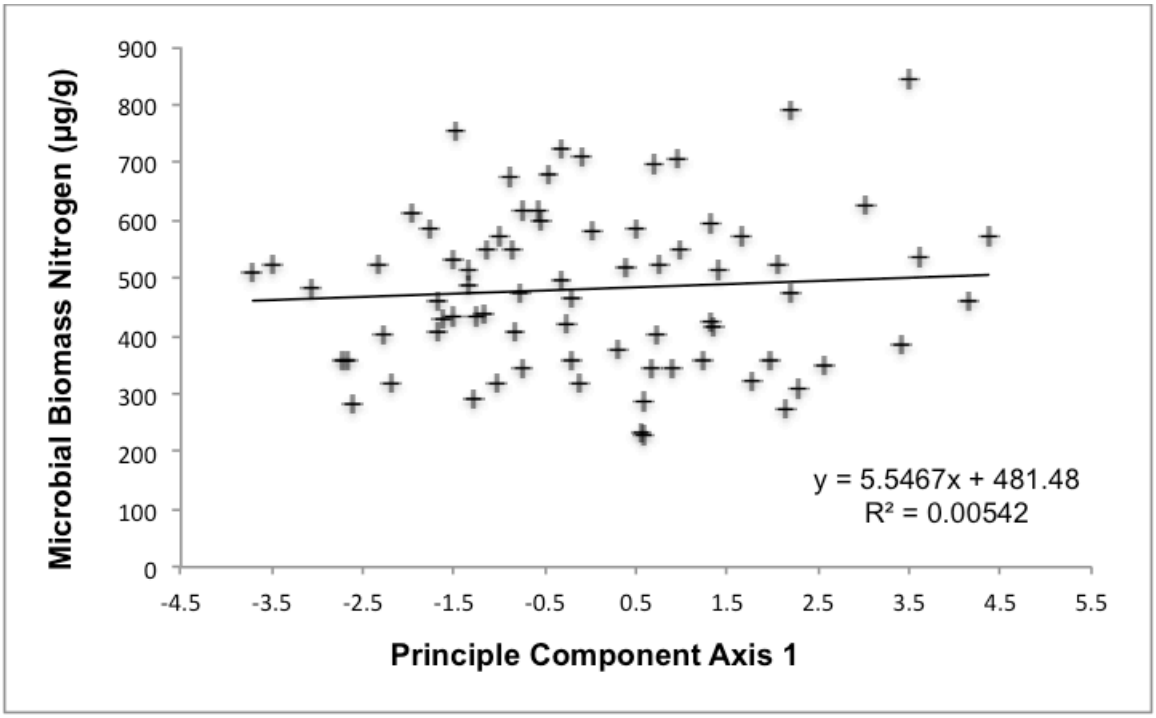

Figure 28. The relationship between microbial biomass nitrogen and PCA1 (soil fertility). Linear regression of microbial biomass nitrogen as predicted by the total principle component analysis axis 1 variation of NMDS-significant physical and chemical soil variables (55.2%); R^2=0.00542, y=5.5467x+481.48, p=0.5301.

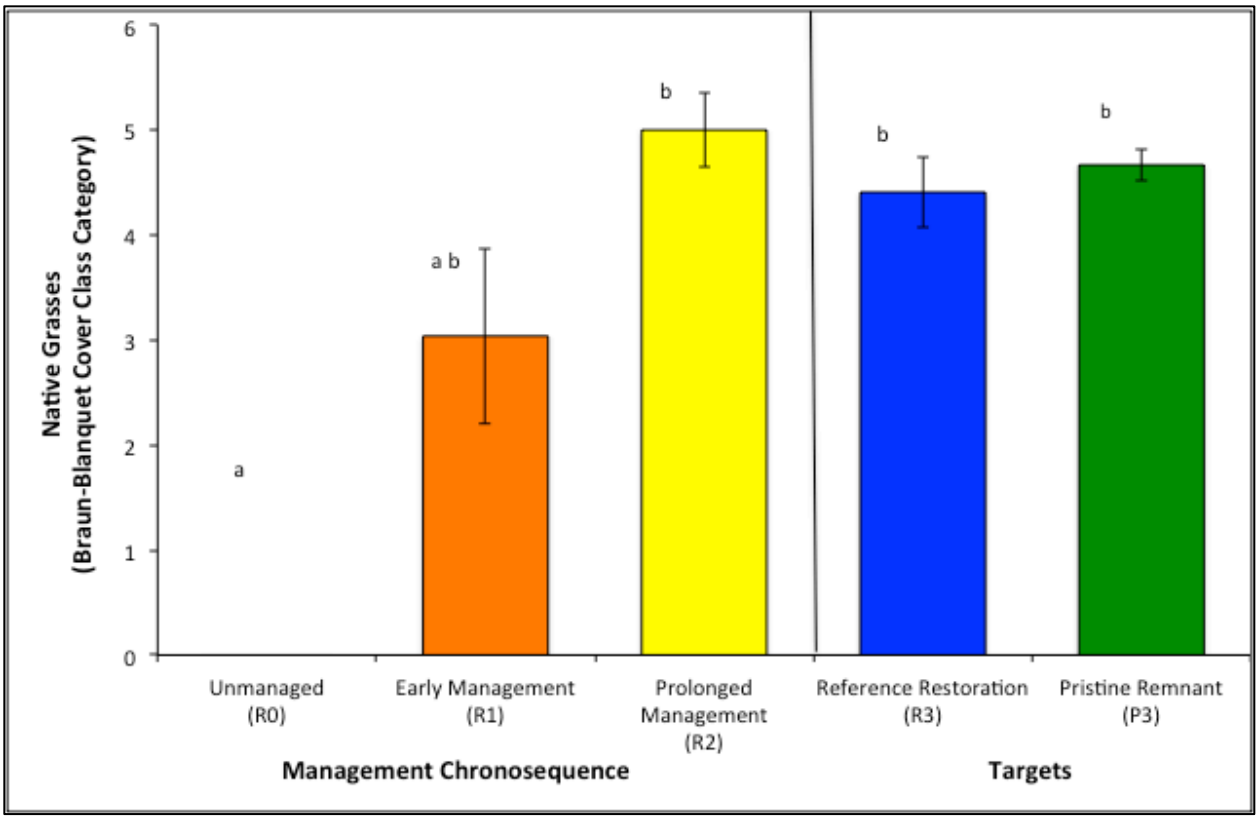

Figure 29. Native grass cover in managed and target prairies. Mean Braun-Blanquet cover class category of native grasses suggests an increase, with increasing duration of management, towards levels found in target prairies (n=15). Braun-Blanquet cover class categories: 0 = not present; 1 = <1% cover; 2 = 1-4% cover; 3 = 5-24% cover; 4 = 25-49% cover; 5 = 50-74% cover; 6 = >75% cover. Vertical bars indicate the standard errors of the mean. Bars with the same letter do not differ significantly at P<0.05 (Tukey's HSD). This baseline vegetation data was obtained from previous study by other CWLMRP scientists. Future study may uncover more robust evidence of the linkages between above- and belowground biota with a larger sample size.

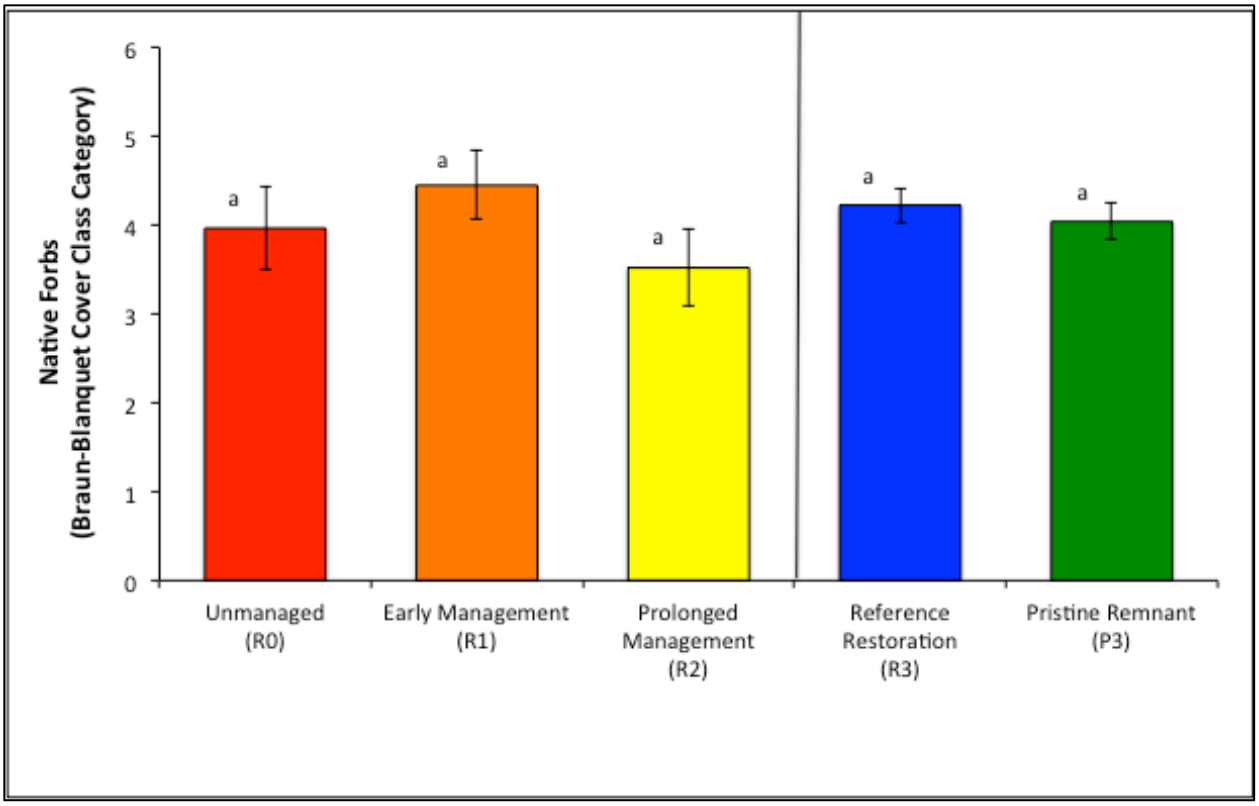

Figure 30. Native forb cover in managed and target prairies. Mean Braun-Blanquet cover class category of native forbs does not demonstrate variation along management sequence nor between target sites (n=15). Braun-Blanquet cover class categories: 0 = not present; 1 = <1% cover; 2 = 1-4% cover; 3 = 5-24% cover; 4 = 25-49% cover; 5 = 50-74% cover; 6 = >75% cover. Vertical bars indicate the standard errors of the mean. Bars with the same letter do not differ significantly at $P<0.05$ (Tukey's HSD). This baseline vegetation data was obtained form previous study by other CWLMRP scientists. Future study may uncover more robust evidence of the linkages between above- and belowground biota with a larger sample size.

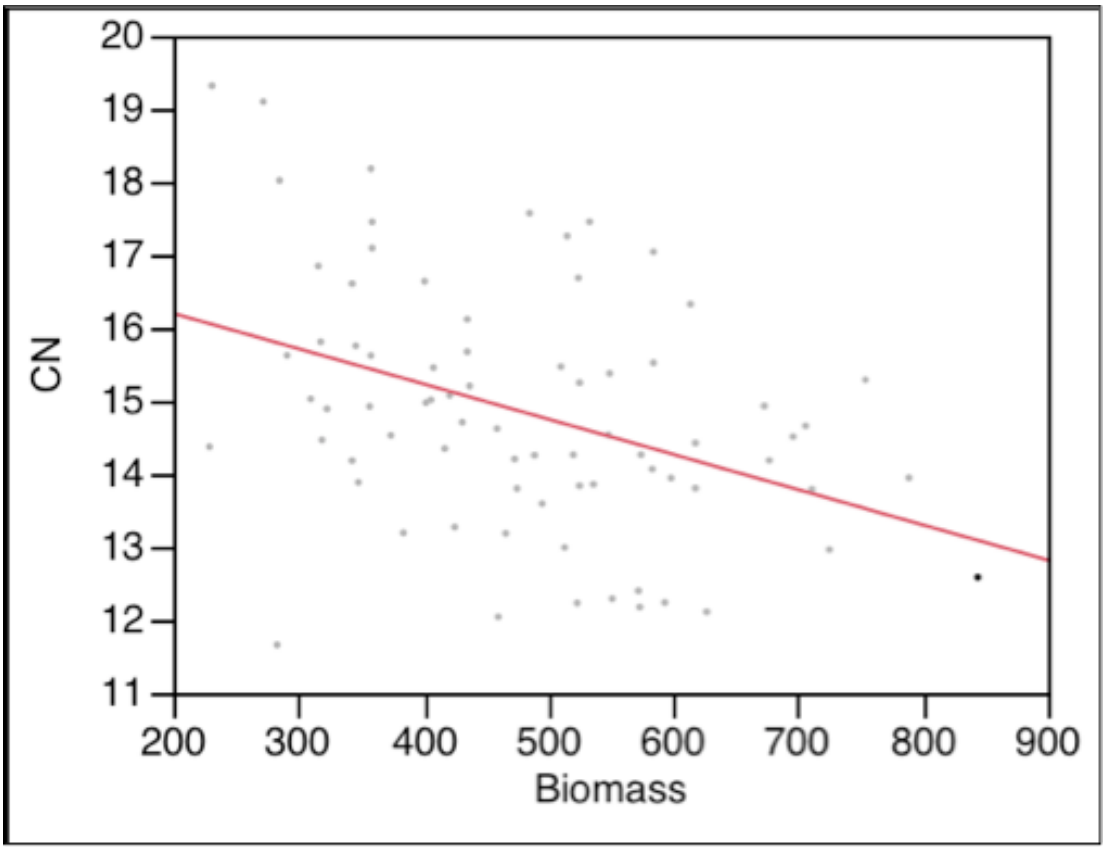

Figure 31. Linear regression plot C:N fit by Biomass in JMP. (p=0.0005, r2=0.1552)

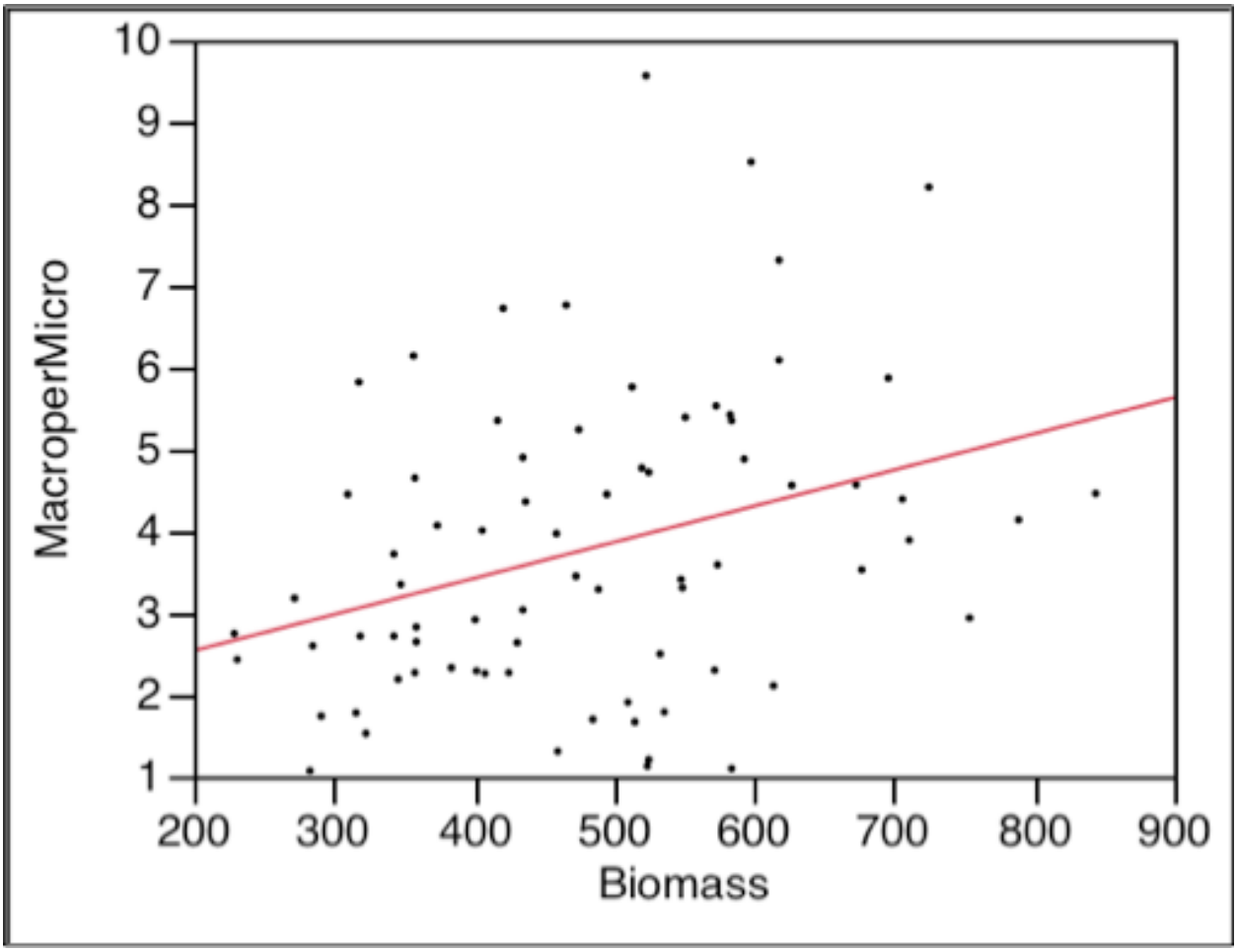

Figure 32. Linear regression plot macroaggregate:microaggregates fit by Biomass.
Analyses performed in JMP. (p=0.0037, r2=0.1098)

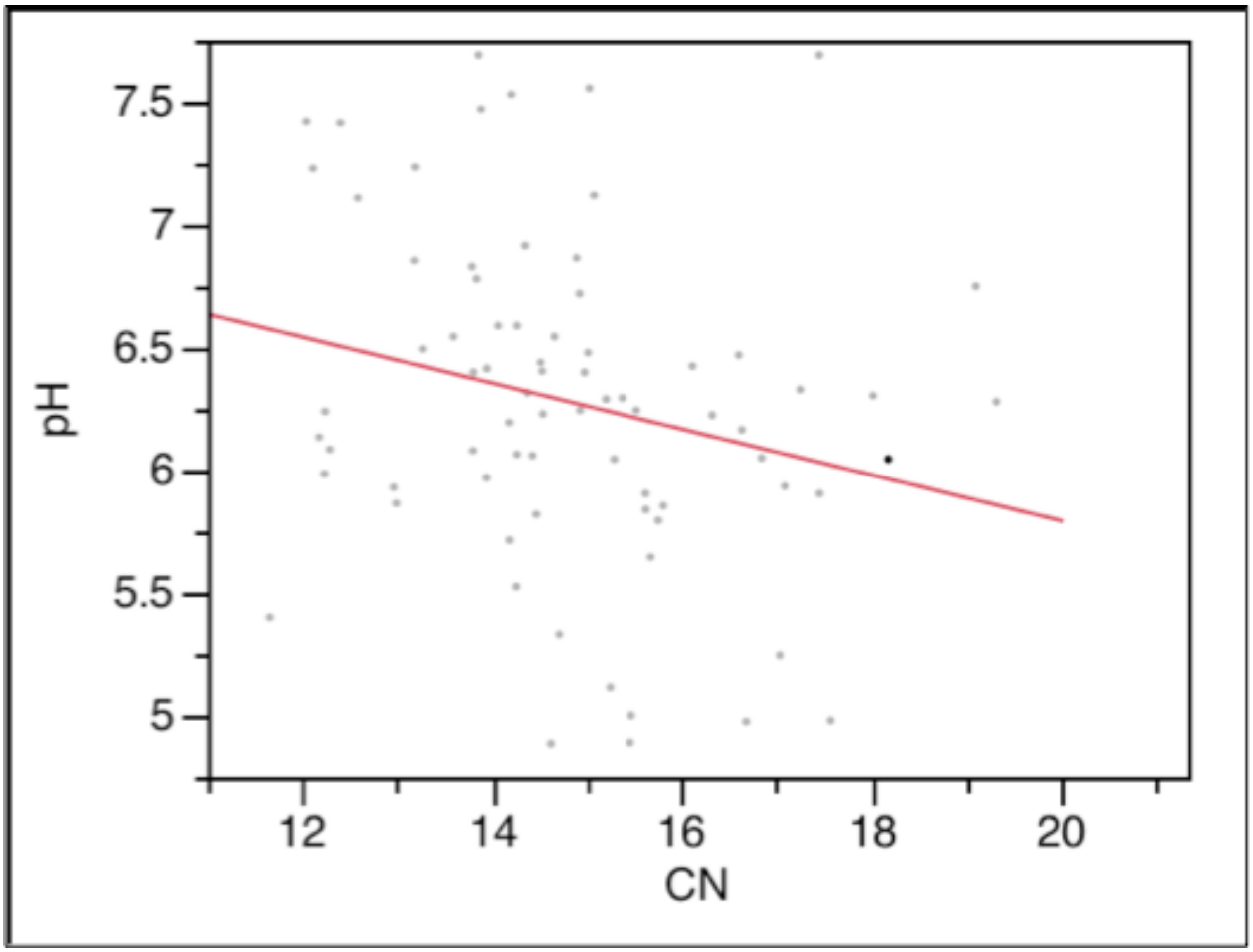

Figure 33. Linear regression plot pH fit by CN. Analyses performed in JMP. (p=0.0416, r2=0.055)

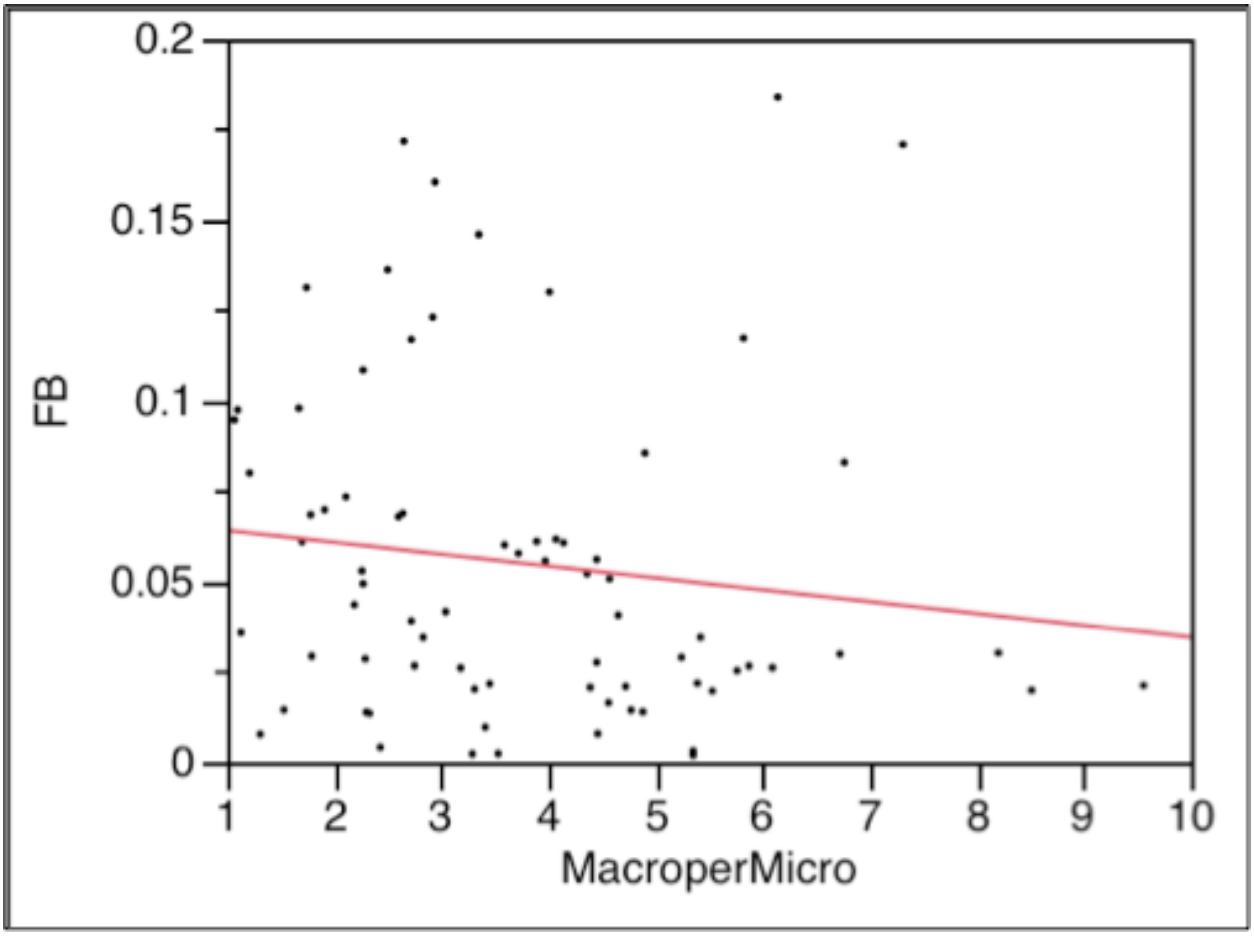

Figure 34. Linear regression plot F:B fit by Macroaggregate:Microaggregate. Analyses performed in JMP. (p=0.2577, r2=0.0175)

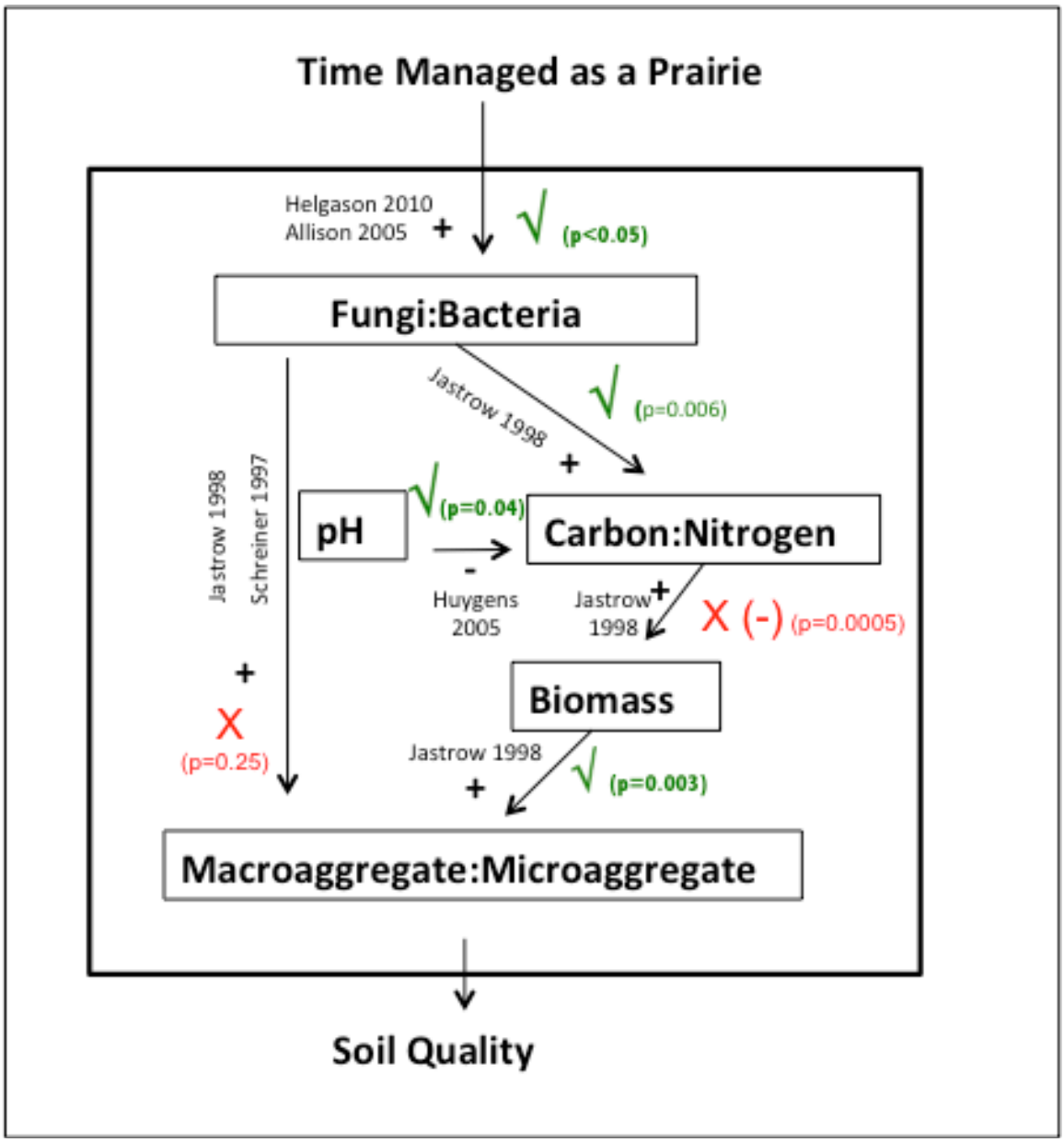

Figure 35. Soil Quality Constituent Interaction Model Revisited. Four of the five proposed interactions between soil quality components were upheld. Positive correlations were found between management duration and fungal:bacterial gene copy ratios (F:B; $p<0.05$), F:B and C:N ($p=0.006$), and between Biomass and Macroaggreage:Microaggregate soil structure ($p=0.003$). Additionally, a slight negative correlation between pH and C:N was found, as expected. Surprisingly, there was and no direct correlation between F:B and Macroaggregate:Microaggregate soil structure. Additionally, C:N was negatively correlated with biomass ($p=0.0005$). Therefore, although biomass was positively correlated with macroaggreage:microaggregate structure ($p=0.003$), the negative relationship between C:N and biomass led to a reversal in the pathway of influence, and a resulting (insignificant) decrease in Macroaggregate:Microaggregate structure with the onset of restoration and management. Linear fit analyses and p-values were obtained using JMP.

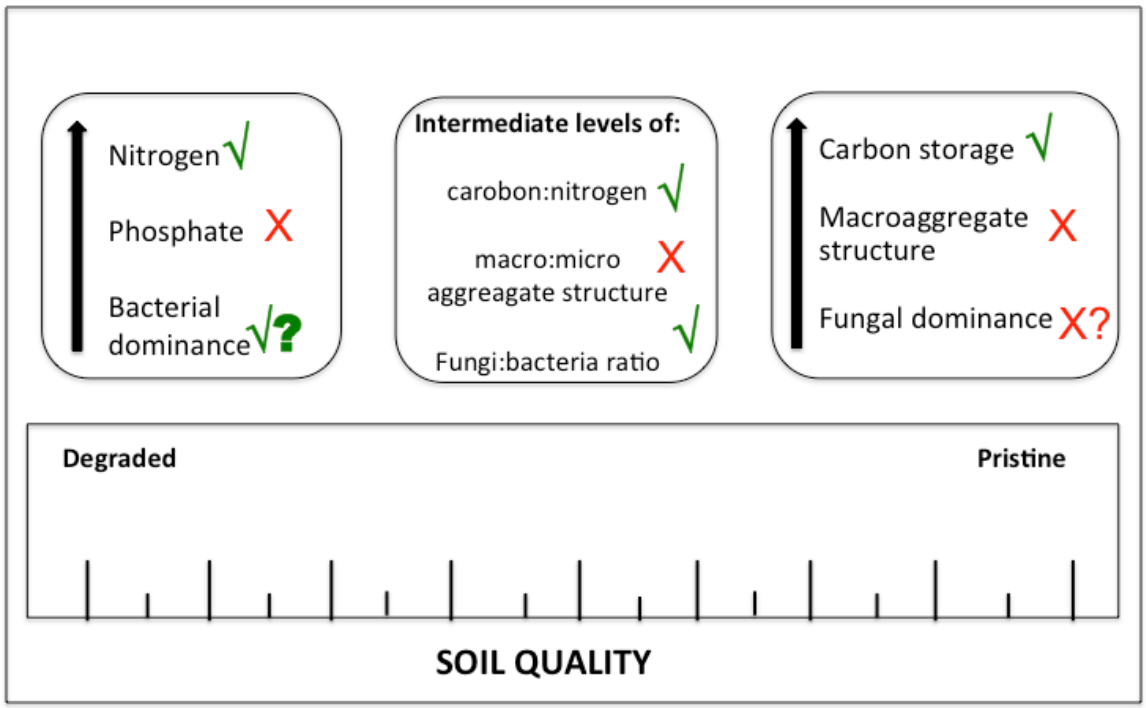

Figure 36. Soil Recovery Model Revisited. This study confirmed greater carbon content in highly developed reference restorations and pristine prairies, and C:N ratios were lowest in degraded old fields, as expected. Surprisingly, phosphate was not a significant soil quality metric, in this study. Although pristine prairies exhibited relatively high levels of macroaggregates, reference restorations did not, and I could not confirm the value of this property, nor the macroaggregate:microaggregate ratio, as indicators of soil quality. While this study suggests relatively high bacteria levels in degraded prairie, as well as relatively high levels of fungi in pristine remnants, these results were not significant. While F:B appears to be an important indicator of soil quality for degraded and early or intermediate-stage restorations, that it may loose some utility towards the very pristine (far right) end of the soil quality gradient.

Tables

Table 1. Study Sites. Name, category, county and location for all research sites. Latitude and Longitude given in decimal degrees (DD)

Site Name	Site Type	Type Code	County	Central Stake Location	
				Latitude (DD)*	Longitude (DD)*
Hawk Hollow Bartlett Road Meadow	Unmanaged Restoration	R0	DuPage	41.954678	-88.187622
Hawk Hollow Northeast	Unmanaged Restoration	R0	DuPage	41.971	-88.1632
Hawk Hollow Northwest	Unmanaged Restoration	R0	DuPage	41.967864	-88.168447
Smith Rd Prairie	Short-term management (<8 years) Restoration	R1	DuPage	41.940028	-88.225472
Spring Brook Prairie Central Fields	Short-term management (<8 years) Restoration	R1	DuPage	41.7393	-88.1836
Bergman Prairie	Short-term management (<8 years) Restoration	R1	Cook	41.669472	-87.905833
Spring Brook Prairie South Fields	Prolonged Management (>8 years) Restoration	R2	DuPage	41.7293	-88.1851
Cuba Marsh	Prolonged Management (>8 years) Restoration	R2	Lake	42.1741	-88.0933
Half Day Field	Prolonged Management (>8 years) Restoration	R2	Lake	42.2111	-87.9332
Glacier Park Pioneer Road South	Model Restoraiton	R3	McHenry	42.430158	-88.289083
Grant Woods	Model Restoraiton	R3	Lake	42.386	-88.126167
Glacier Park Pioneer Road North	Model Restoraiton	R3	McHenry	42.435164	-88.292367
Larson Prairie	Model Remnant	P3	McHenry	42.191922	-88.301783
Somme Prairie	Model Remnant	P3	Cook	42.141306	-87.838389
Wadsworth Prairei	Model Remnant	P3	Lake	42.4396	-87.9287

Table 2. Baseline Soil Properties. Baseline data for soil texture and phosphorous were collected by the Chicago Wilderness Land Management Research Program in 2009.

Site Name	Site Type Code	Soil Texture		Phosphorous ([µg/cm3] per 28 days)
		Baseline calculated from initial CWLMRP assessment (2009)	Based on Web Soil Survey (2012)	
Hawk Hollow Bartlett Road Meadow	R0	Silty Clay	silt loam (77%), silty clay loam (23%)	7.6
Hawk Hollow Northeast	R0	Silt loam	silt laom (~80%), silty clay loam (19%)	5.4
Hawk Hollow Northwest	R0	Clay loam	silt loam (74%), silty clay loam (23%), loam (3%)	7.4
Smith Rd Prairie	R1	Silty Clay	silt loam (86%), silty clay loam (14%)	17.4
Spring Brook Prairie Central Fields	R1	Silty Clay loam	silty clay loam (52%), silt loam (45%)	20.4
Bergman Prairie	R1	loam	Silt loam	10.2
Spring Brook Prairie South Fields	R2	Silt loam	silty clay loam (75%), silt loam (24%)	26.8
Cuba Marsh	R2	Silty Clay loam	silt loam (88%), silty clay loam (15%)	23.7
Half Day Field	R2	Silty Clay loam	silt loam (54%), silty clay loam (46%)	20.5
Glacier Park Pioneer Road South	R3	Silty Clay loam	silt loam (51%), loam (49%)	27.9
Grant Woods	R3	Sandy loam	silt loam (47%), muck (30%), silty clay loam (~23%)	1.8
Glacier Park Pioneer Road North	R3	Silty Clay loam	silt loam (84%), silty clay loam (15.2%)	37.9
Larson Prairie	P3	Silt loam	silty clay loam, loam, clay loam, muck	2.1
Somme Prairie	P3	Sandy loam	Sandy loam	1.6
Wadsworth Prairie	P3	Silt loam	Silt loam, silty clay loam	1.0

Table 3. Sampling dates. All samples were collected within three weeks in mid-July, 2012.

Site Name	Site Type Code	Field Sampling Trips	
		Gregorian Date	Julian Date
Smith Rd Prairie	R1	9-Jul-12	12191
Cuba Marsh	R2	11-Jul-12	12193
Half Day Field	R2	11-Jul-12	12193
Larson Prairie	P3	11-Jul-12	12193
Wadsworth Prairie	P3	14-Jul-12	12196
Glacier Park Pioneer Road South	R3	15-Jul-12	12197
Grant Woods	R3	15-Jul-12	12197
Glacier Park Pioneer Road North	R3	15-Jul-12	12197
Hawk Hollow Bartlett Road Meadow	R0	21-Jul-12	12203
Hawk Hollow Northeast	R0	21-Jul-12	12203
Hawk Hollow Northwest	R0	21-Jul-12	12203
Spring Brook Prairie Central Fields	R1	21-Jul-12	12203
Bergman Prairie	R1	21-Jul-12	12203
Spring Brook Prairie South Fields	R2	21-Jul-12	12203
Somme Prairie	P3	27-Jul-12	12209

Table 4. Fungal and Bacterial Gene Copy Numbers. Quantitative microbial community analysis using qPCR to estimate the copy number of SSU 18S rRNA in fungi and SSU 16S rRNA in bacteria under different management regimes.

Site (Abbreviation)	Fungi (Copy numbers x 10^8 g^{-1} soil ± 1 s.e.)	Bacteria (Copy numbers x 10^{10} g^{-1} soil ± 1 s.e.)
Unmanaged (R0)	2.34 ± 0.39	1.33 ± 0.09
Early Management (R1)	3.03 ± 0.38	0.53 ± 0.08
Prolonged Management (R2)	7.19 ± 1.25	0.67 ± 0.09
Reference Restoration (R3)	4.54 ± 0.56	1.43 ± 0.21
Pristine Remnant (P3)	7.24 ± 1.83	1.88 ± 0.21

Table 5. Influences of abiotic soil properties to microbial community structure and abundance. Significant abiotic environmental vectors influencing the microbial community assembly in unmanaged, managed, and target prairie sites. R^2 values are significant at: '***' = $p < 0.001$; '**' = $p < 0.01$; '*' = $p < 0.05$.

Soil Factor	**R^2**	**Pr (>r)**
% carbon	0.449	9.99e-05 ***
% silt	0.412	9.99e-05 ***
Total % nitrogen	0.394	9.99e-05 ***
% sand	0.348	9.99e-05 ***
pH	0.3368	9.999e-05 ***
Gravimetric moisture	0.159	0.001 **
Bulk density	0.061	0.103
Available phosphate	0.040	0.219
% clay	0.022	0.438
Ratio of macroaggregates: microaggregates	0.018	0.5313

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Appendix: Supplementary Materials

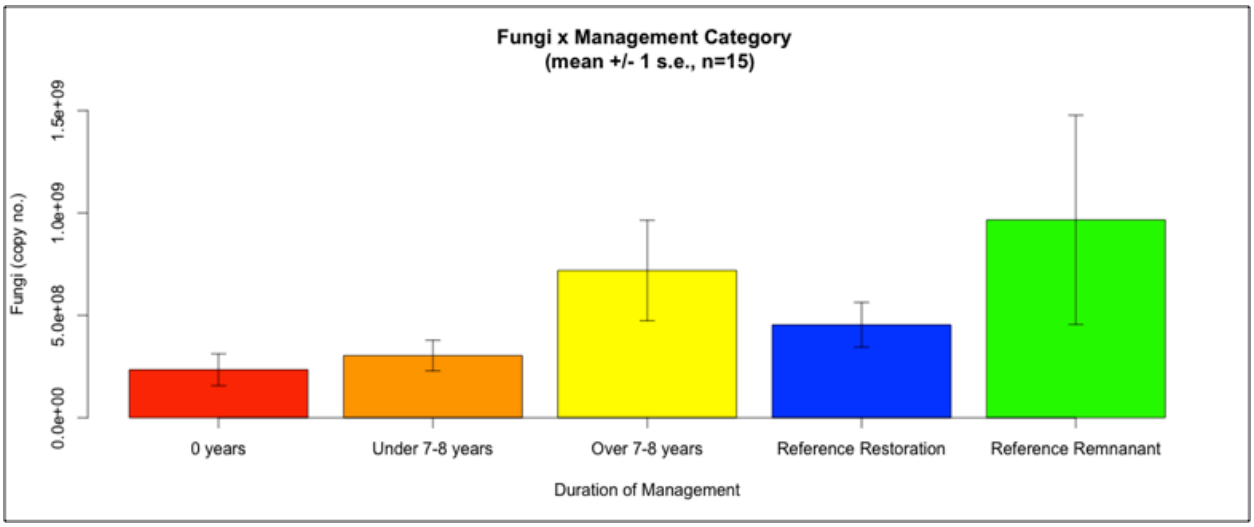

Figure 37. Fungal gene copy ratio in managed and target prairies.

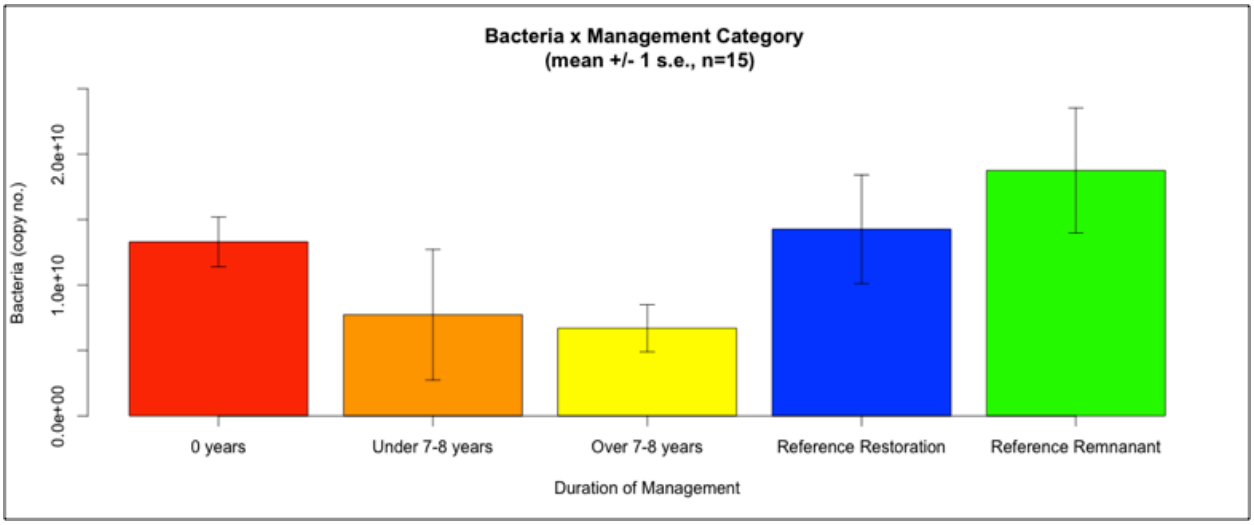

Figure 38. Bacterial gene copy ratio in managed and target prairies.

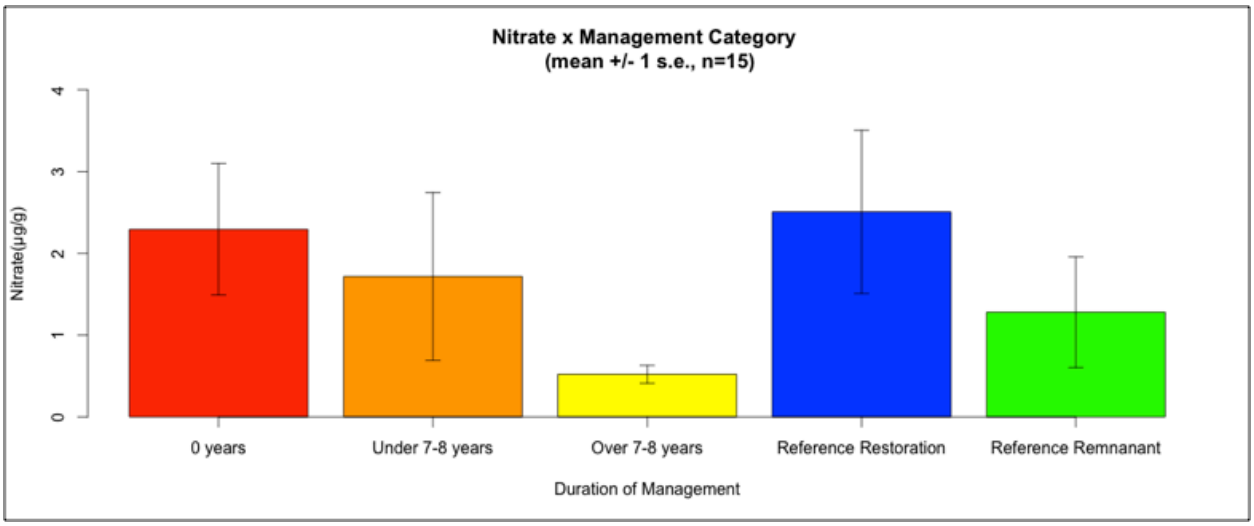

Figure 39. Microbial nitrate in managed and target prairies.

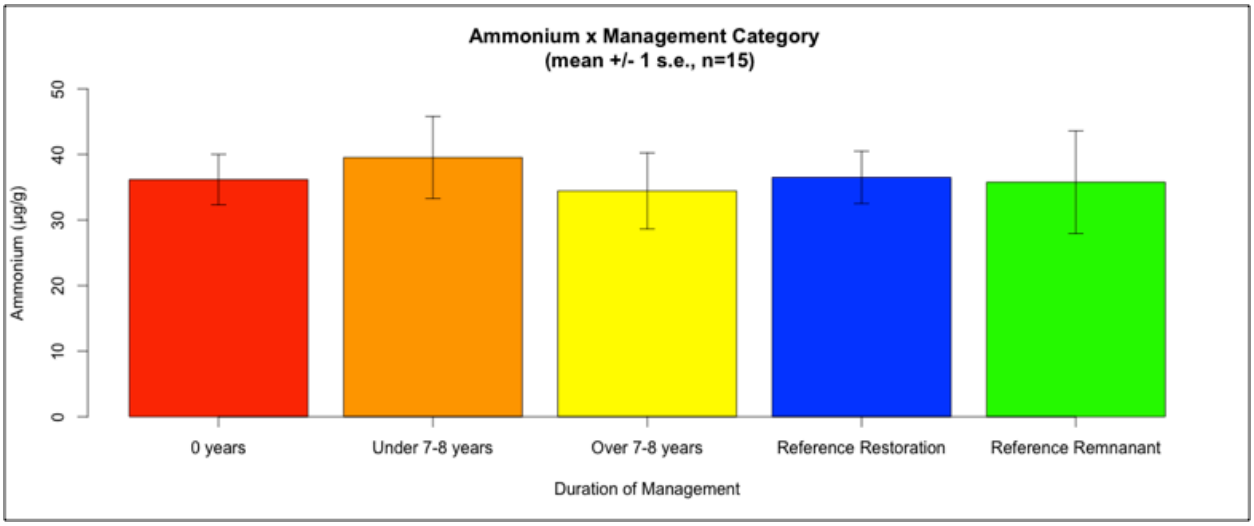

Figure 40. Microbial ammonium in managed and target prairies.

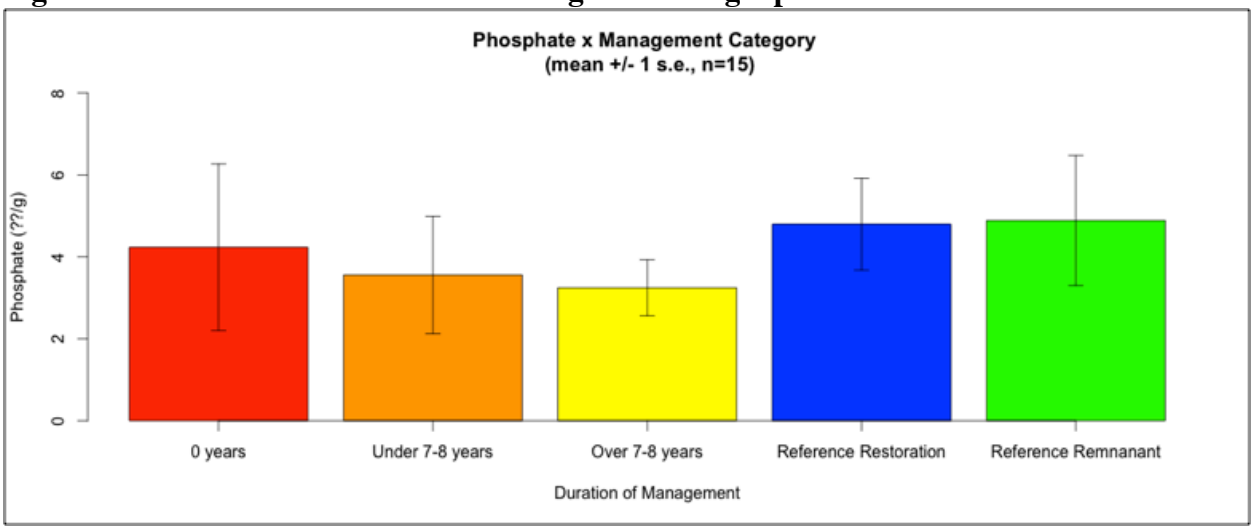

Figure 41. Phosphate in managed and target prairies.

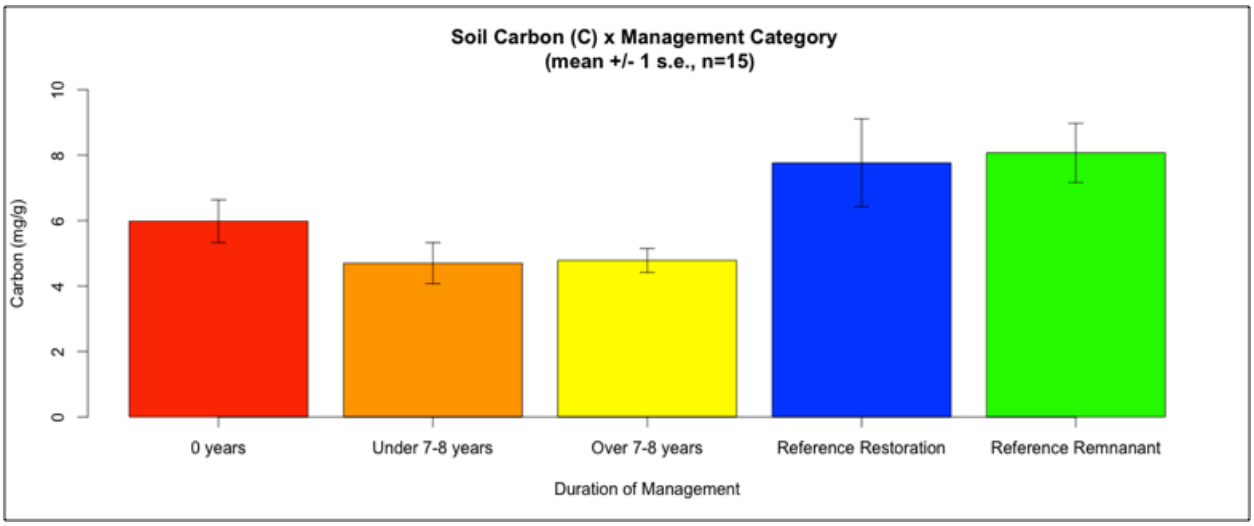

Figure 42. Total soil carbon in managed and target prairies.

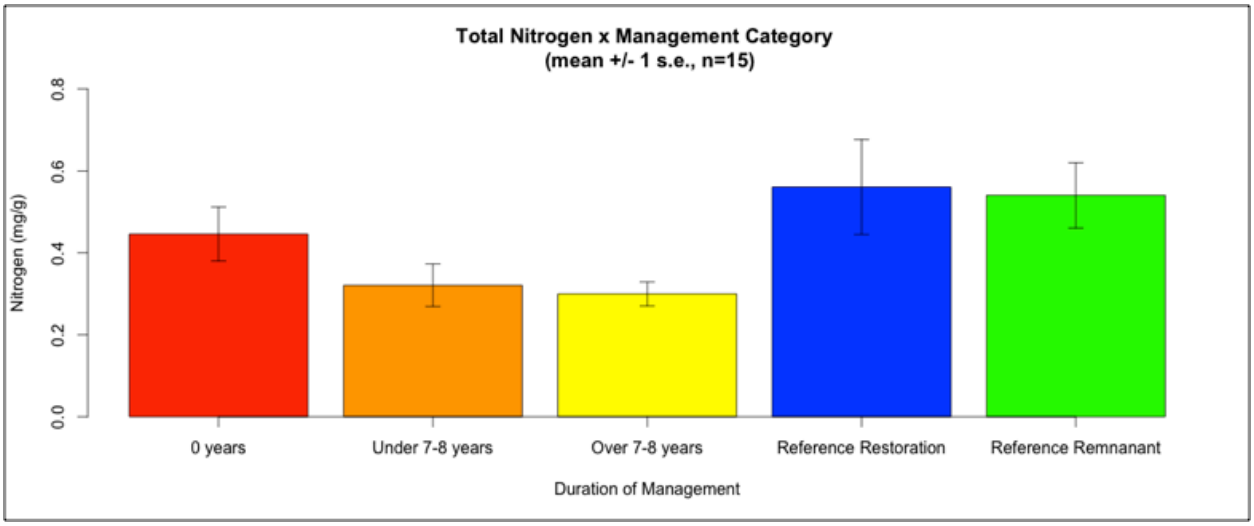

Figure 43. Total soil nitrogen in managed and target prairies.

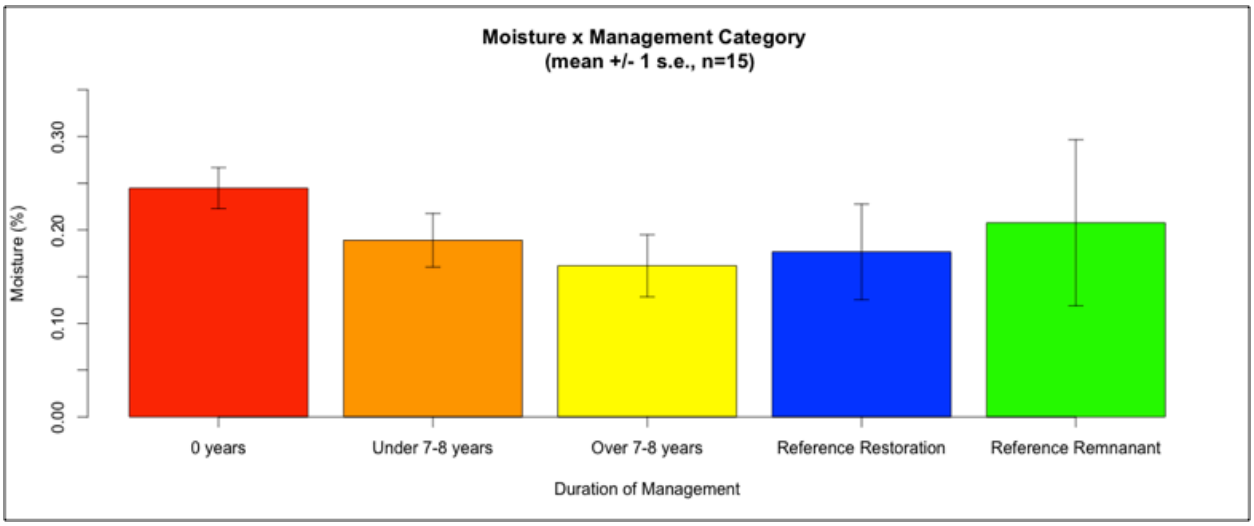

Figure 44. Gravimetric moisture content in managed and target prairie soils.

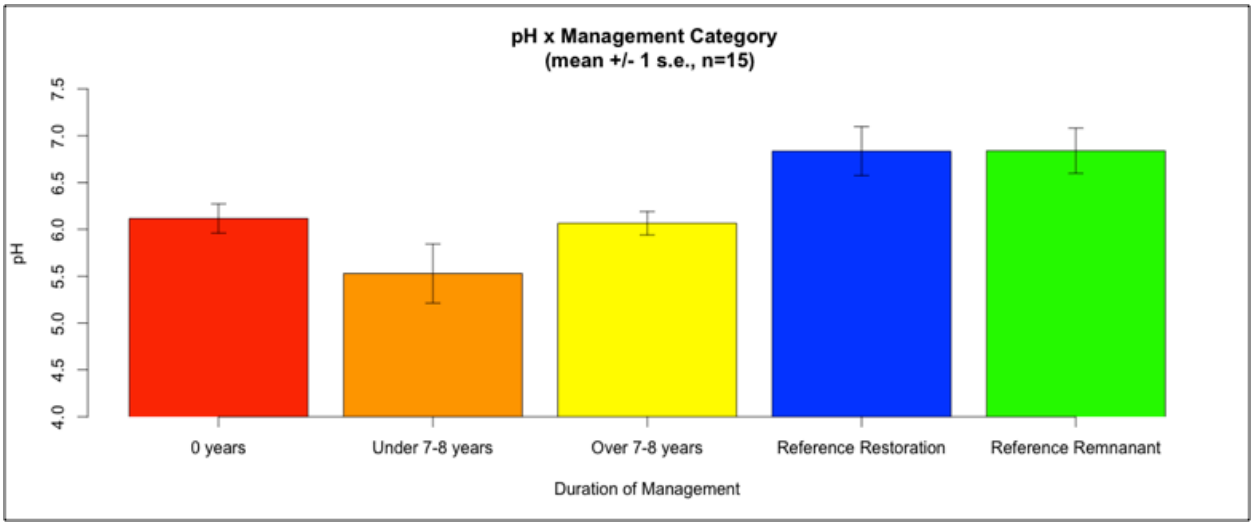

Figure 45. Soil pH in managed and target prairies.

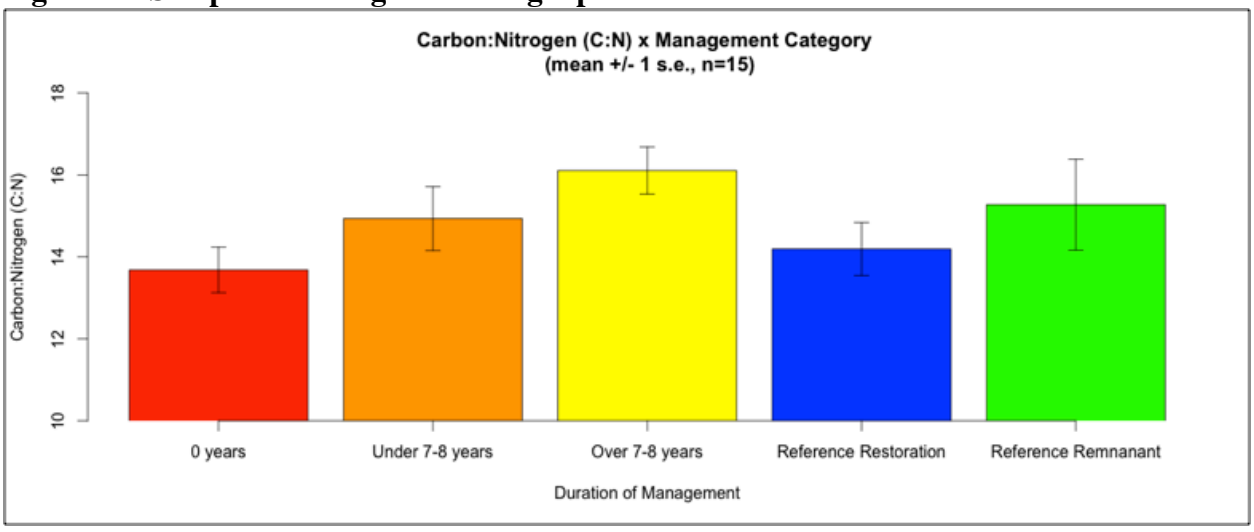

Figure 46. C:N ratios in managed and target prairies.

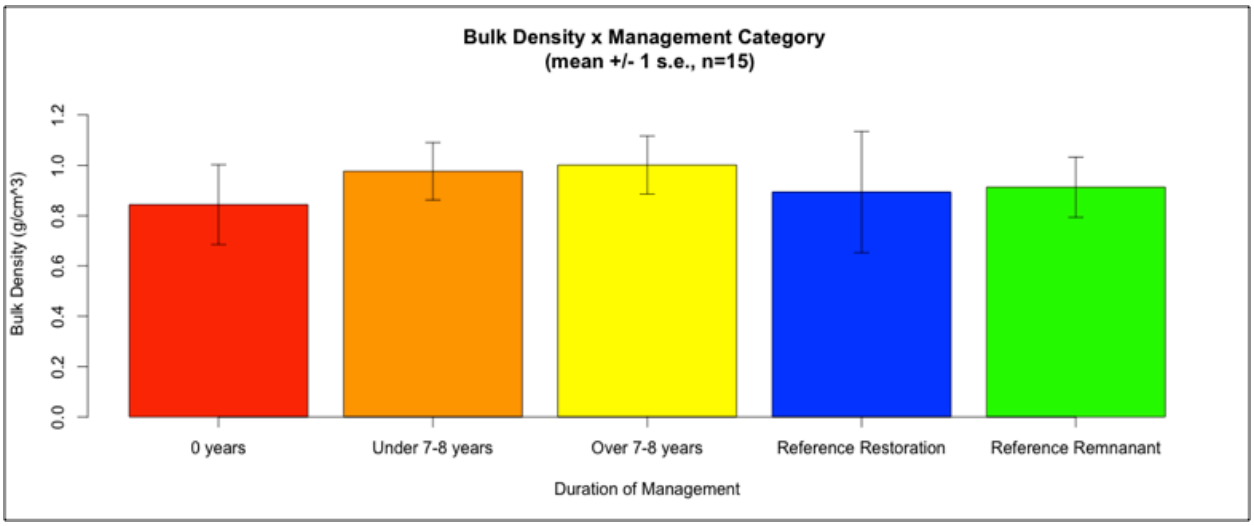

Figure 47. Bulk density in managed and target prairies.

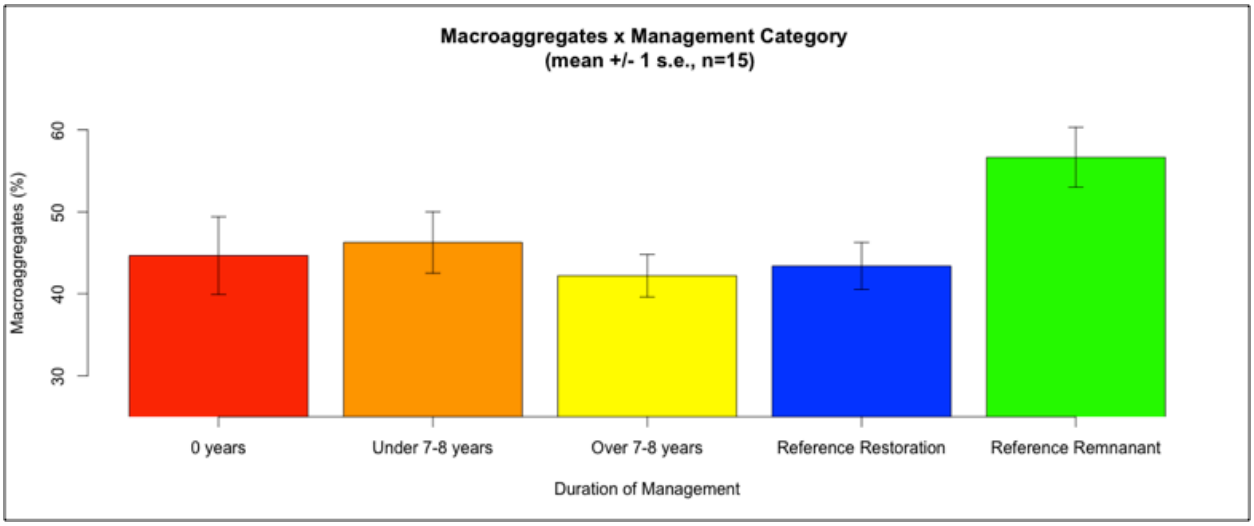

Figure 48. Water-stable macroaggregtes in managed and target prairies.

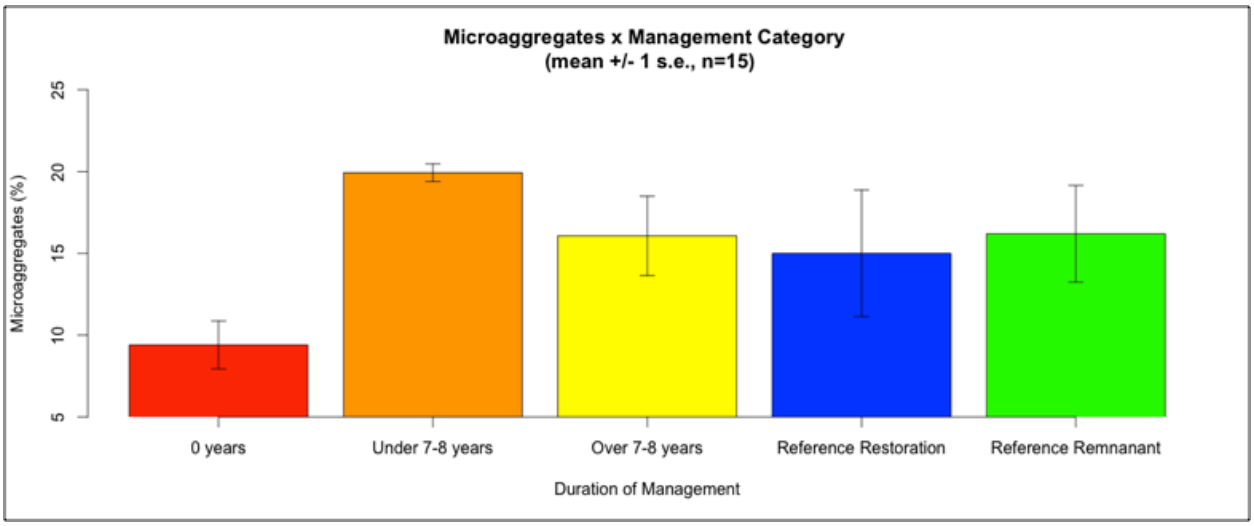

Figure 49. Water-stable microaggregates in managed and target prairies.

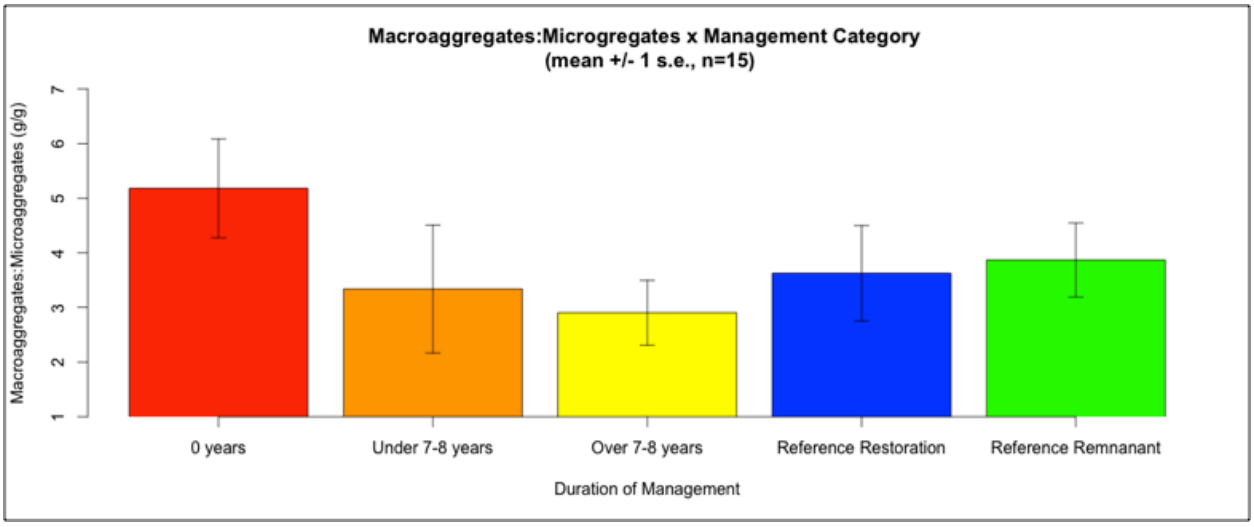

Figure 50. Water-stable macroaggregte:microaggregate ratios in managed and target prairies.

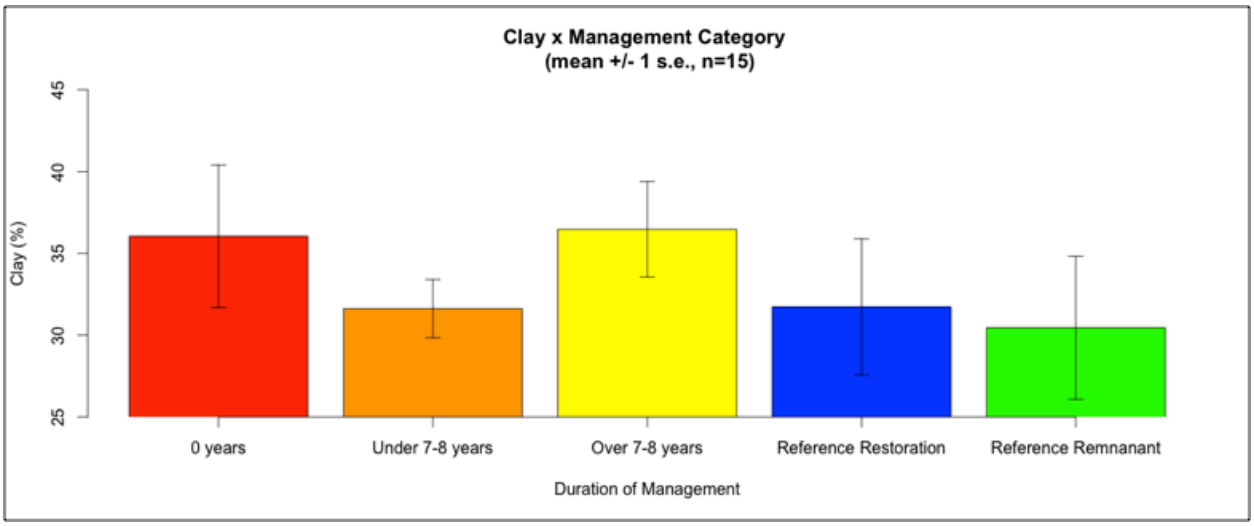

Figure 51. Clay content in managed and target prairie soils.

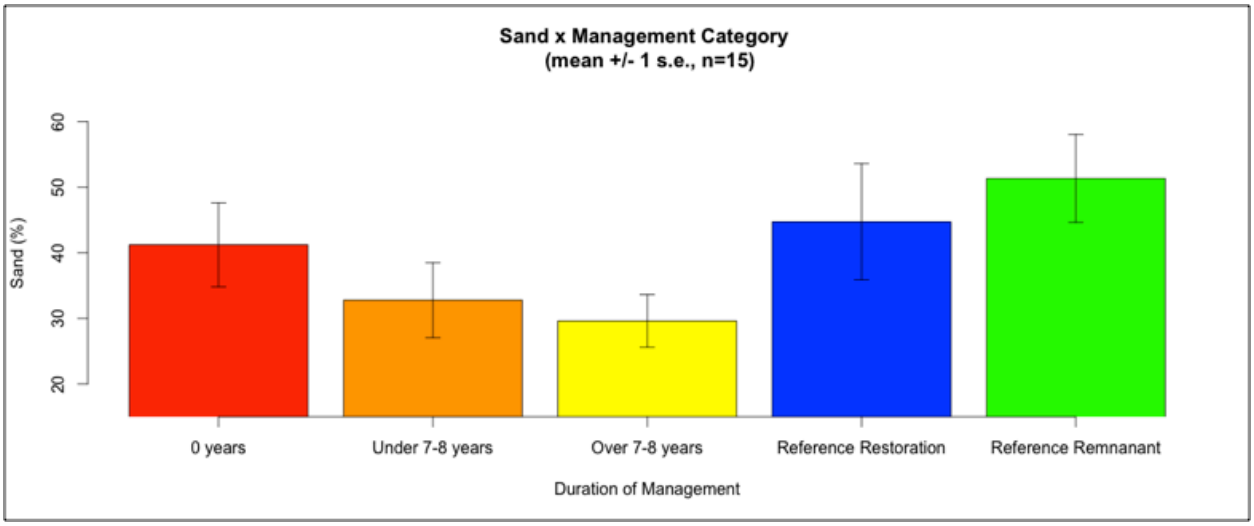

Figure 52. Sand content in managed and target prairie soils.

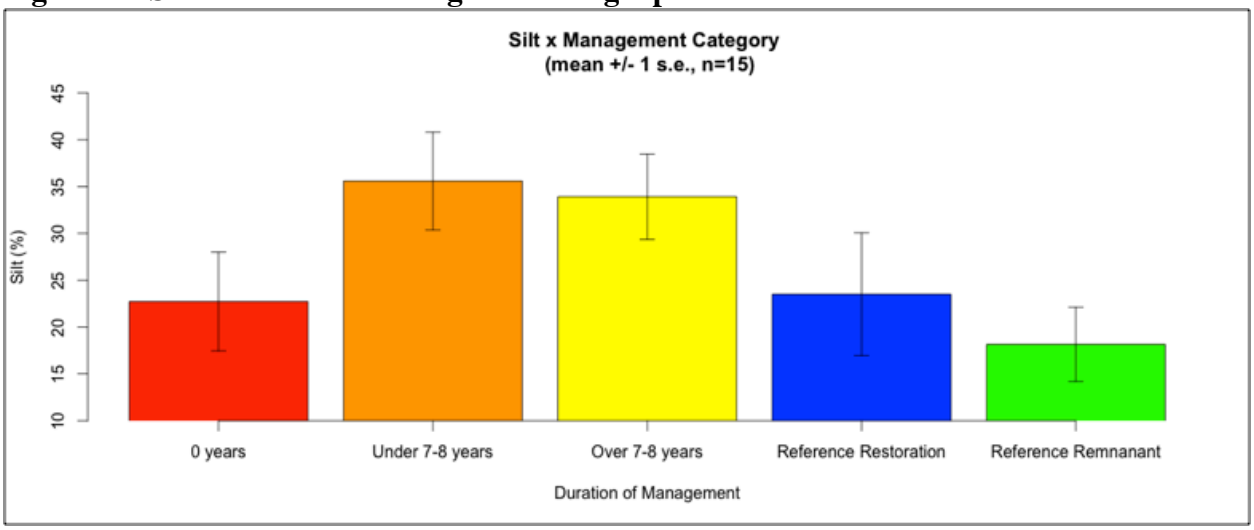

Figure 53. Silt content in managed and target prairie soils.

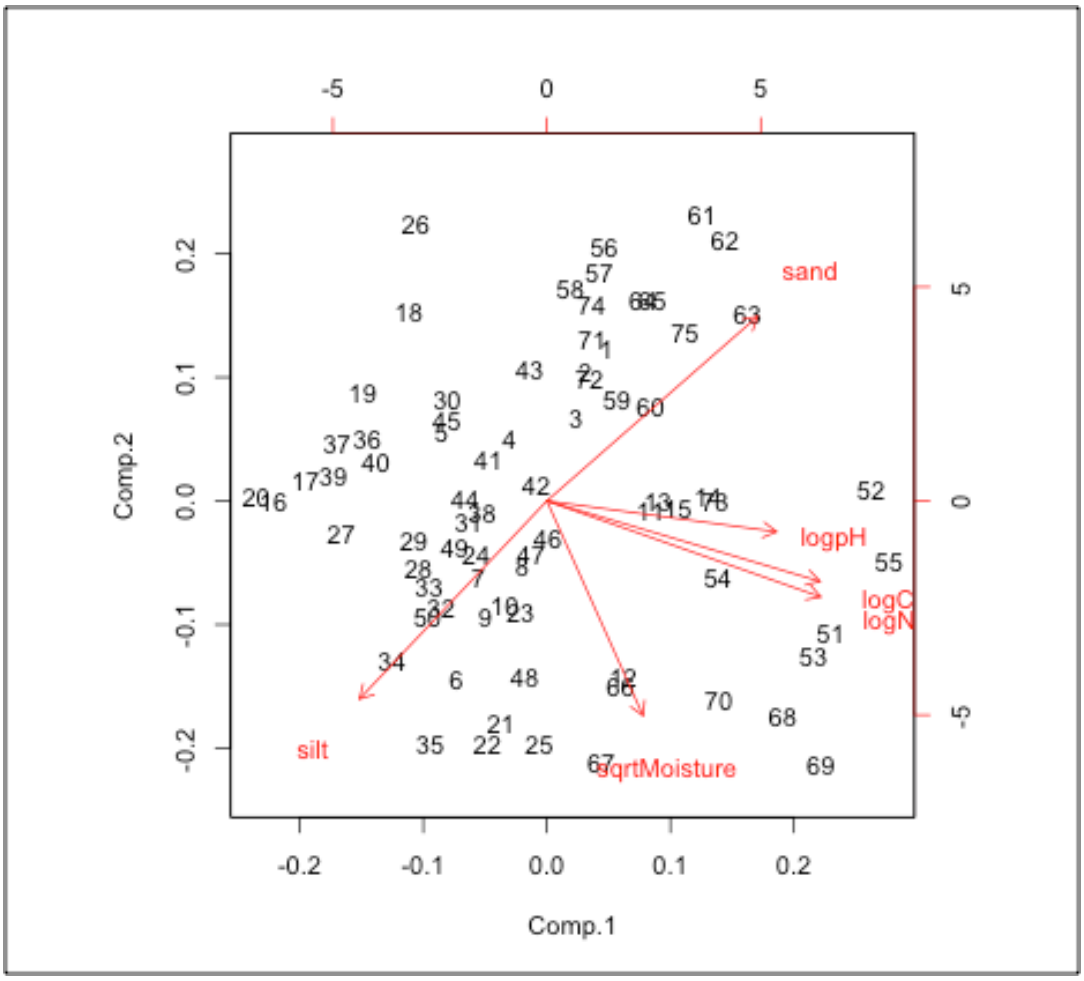

Figure 54. PCA Biplot. Texture & moisture varying strongly across both Comp. 1 & 2. logC, logN, logpH varying mostly across Comp. 1. Data transformed.

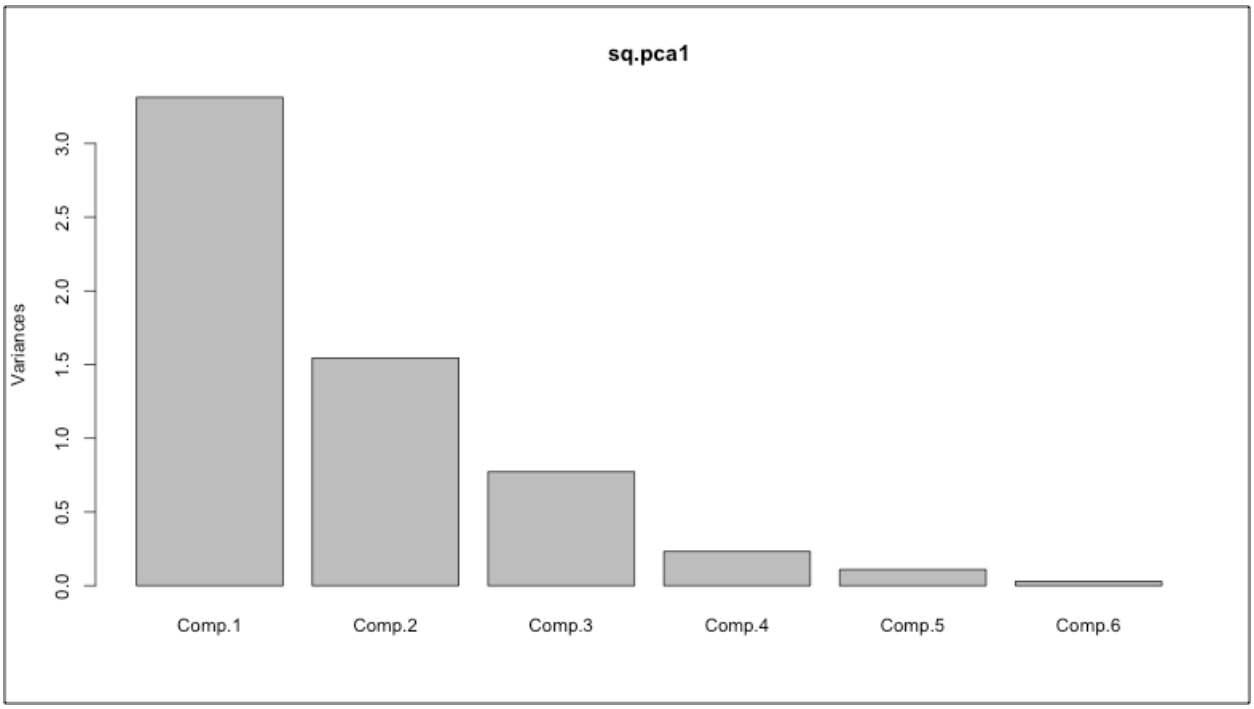

Figure 55. PCA Scree Plot (all sq indicators except F, B, Biomass). Dropoff after Comp. 1. Data transformed: sand, silt, logpH, logC, logN, sqrtMoisture

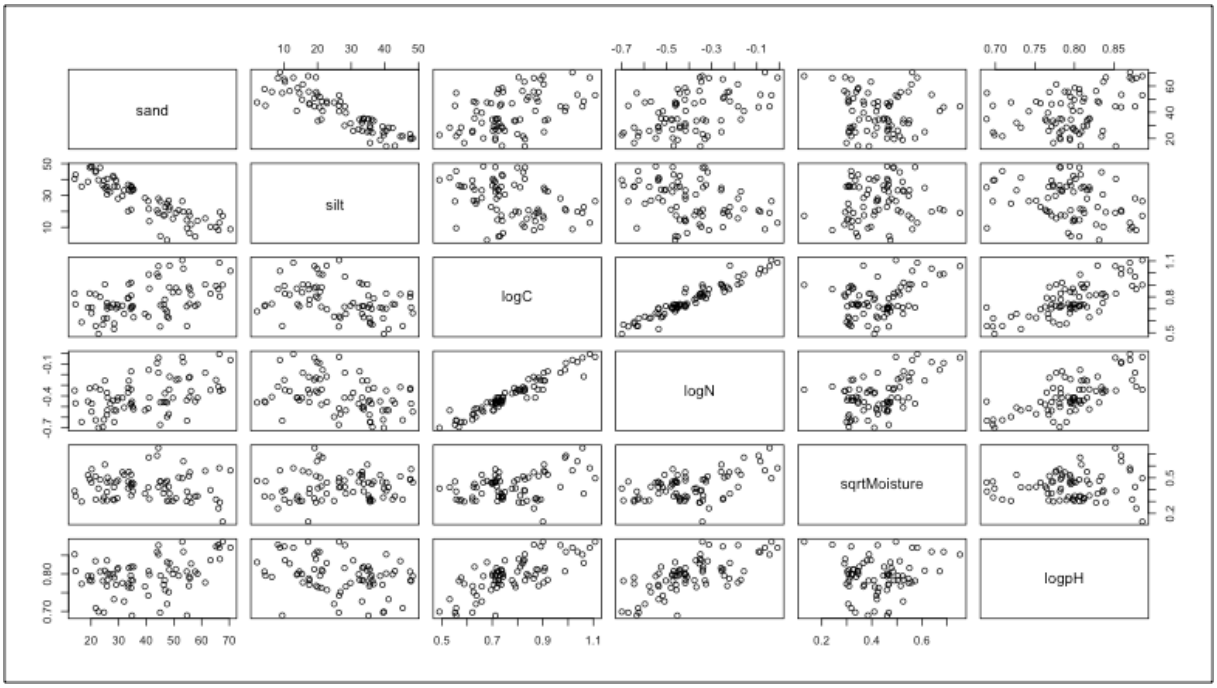

Figure 56. Pairwise scatterplots. Silt and sand, log C & log correlated. Other variables plotted in chart: log N, square root moisture, log pH.

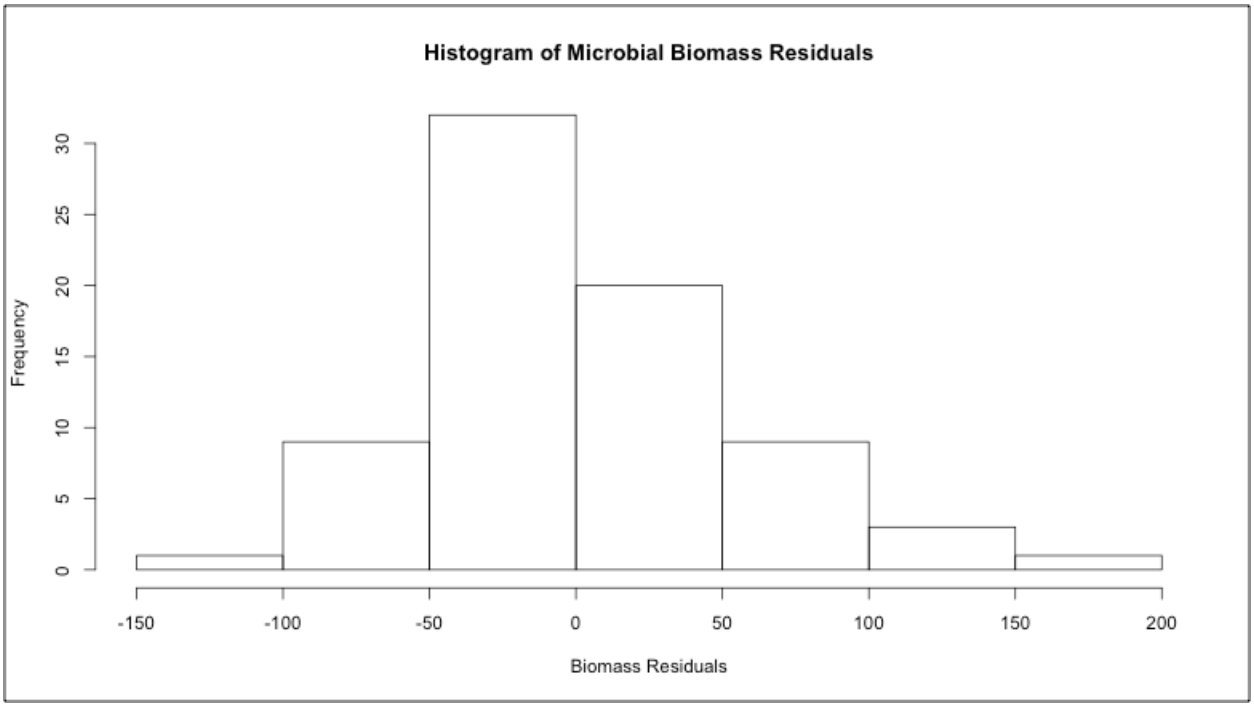

Figure 57. Histogram of Microbial Biomass Residuals in Managed and Reference/Pristine Prairies. Slight positive skew.

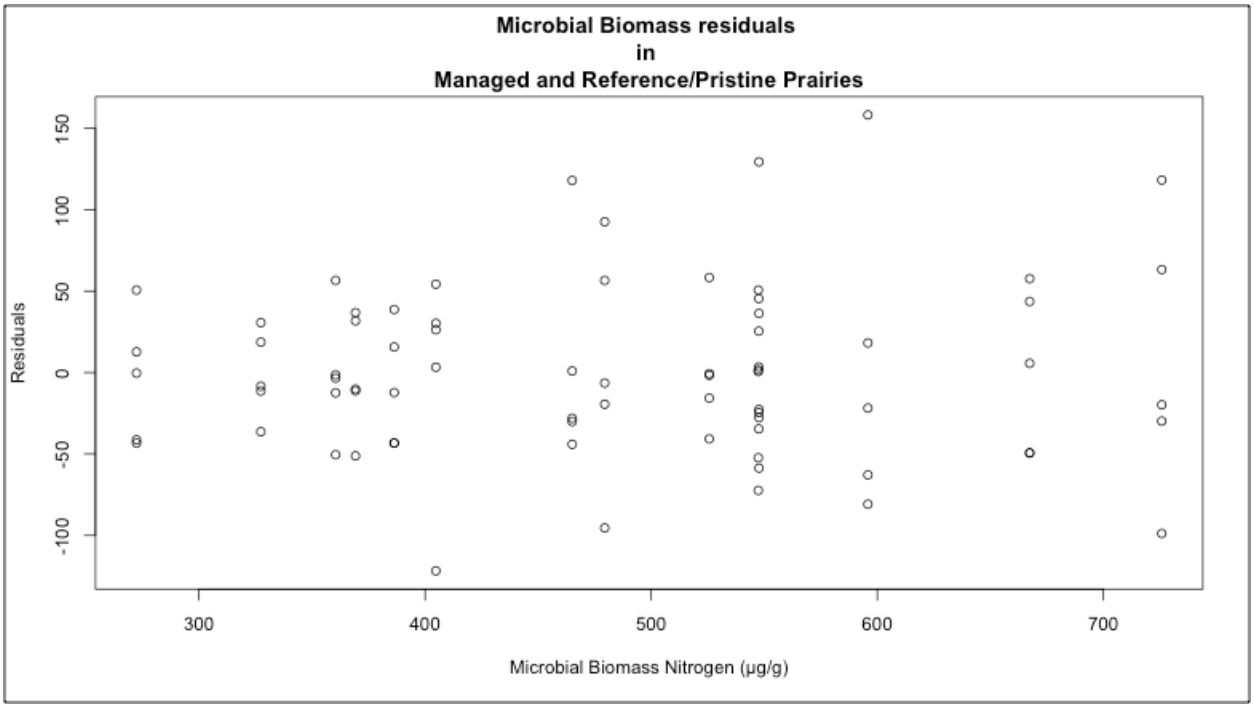

Figure 58. Microbial Biomass Residuals in Managed and Reference/Pristine Prairies.

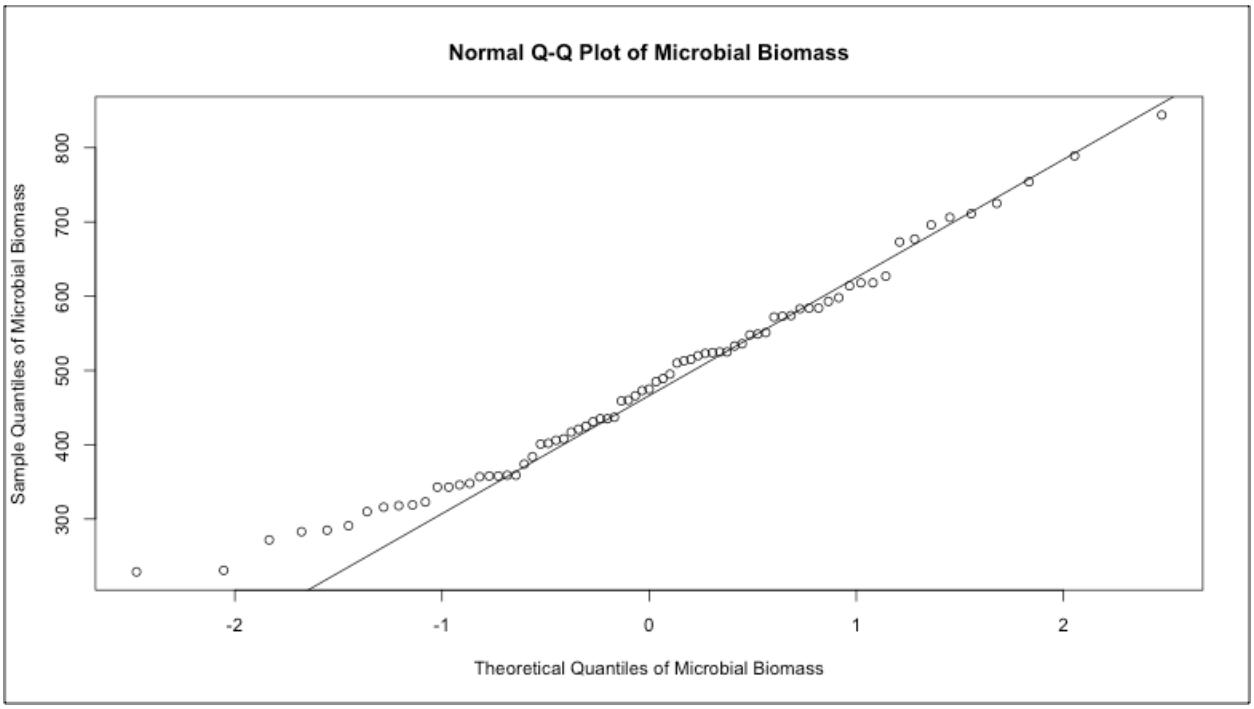

Figure 59. QQ-plot of microbial biomass in managed and reference/pristine prairies. No major deviation from normal

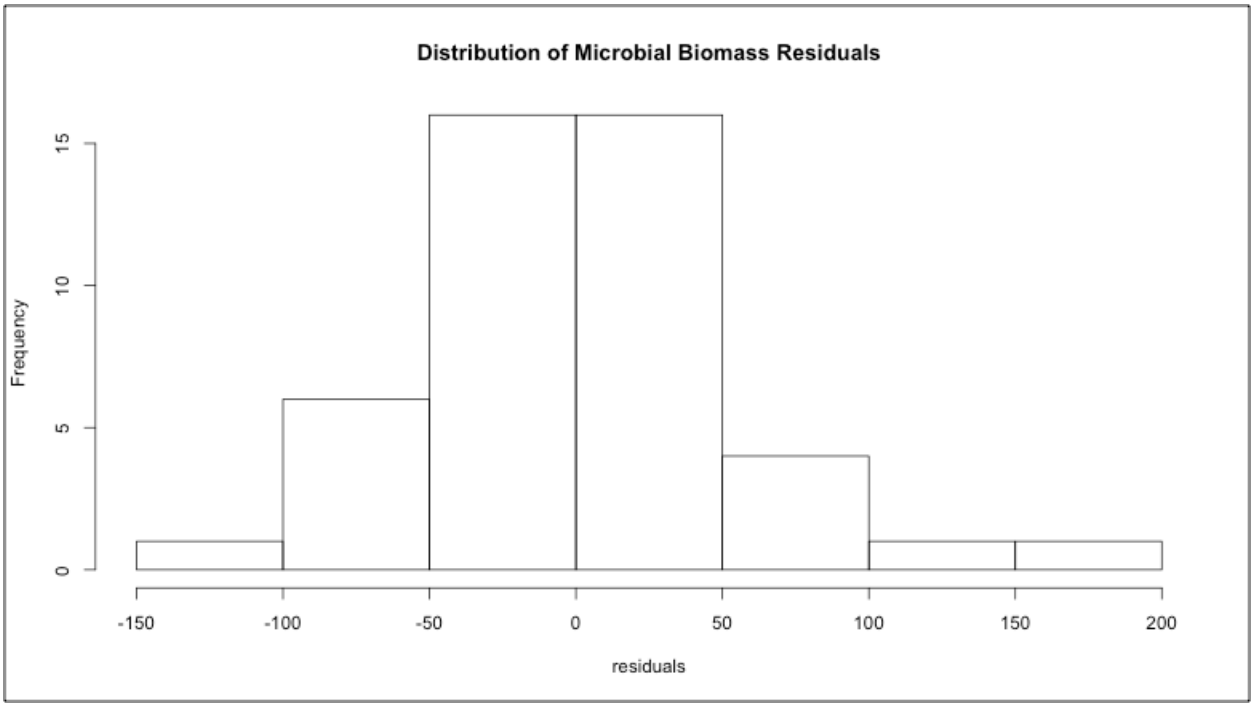

Figure 60. Histogram of Microbial Biomass Residuals across Management Chronosequence.

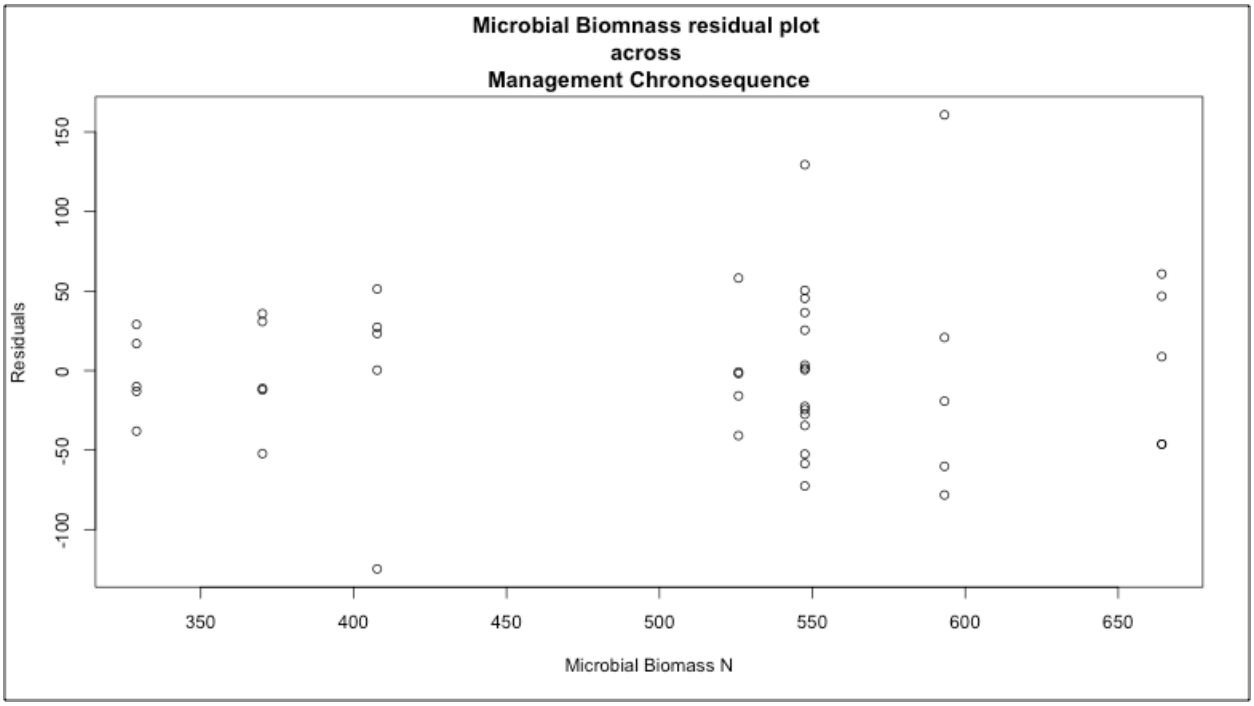

Figure 61. Microbial Biomass Residuals across Management Chronosequence.

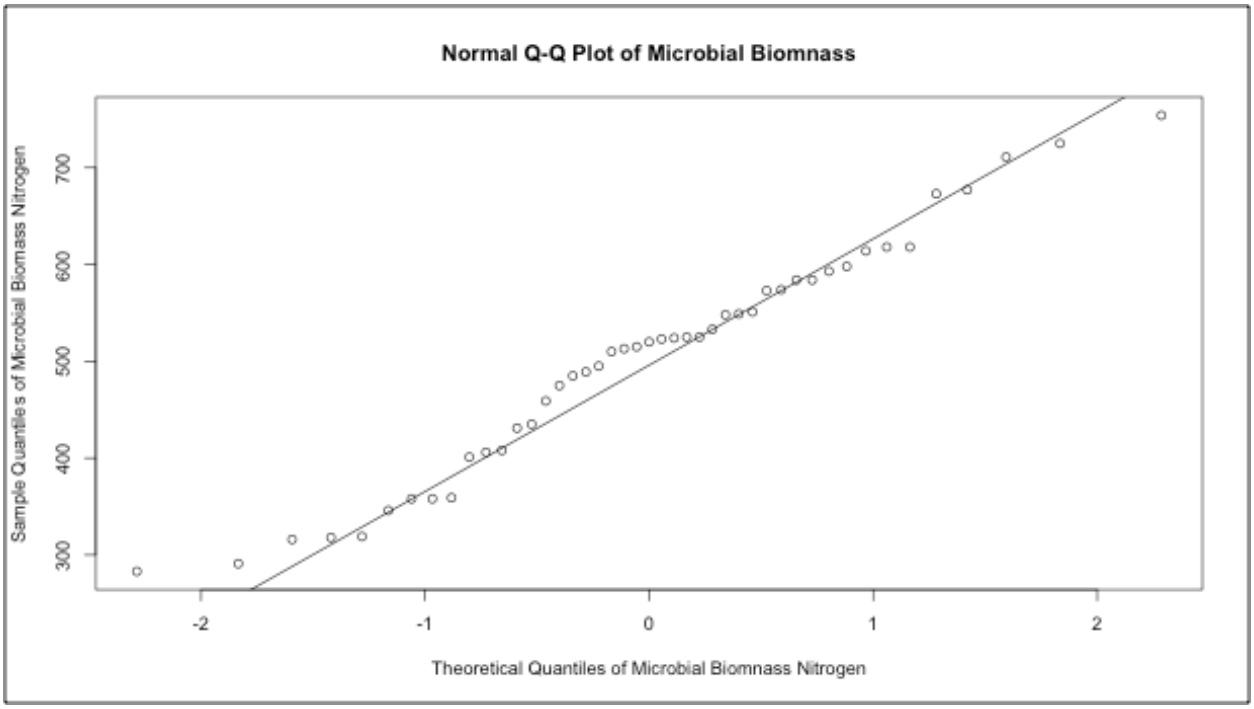

Figure 62. Q-Q Plot of Microbial Biomass across Management Chronosequence. No major deviation from normal.

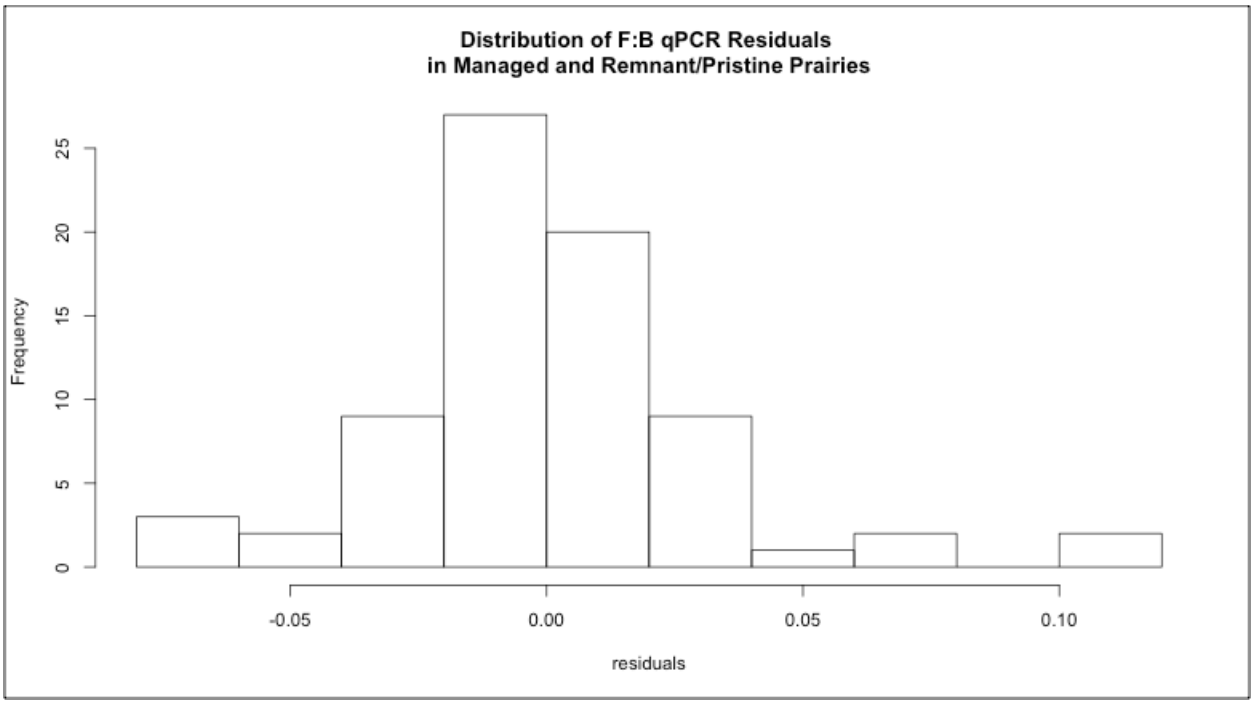

Figure 63. Histogram of F:B gene copy ratio residuals in managed and reference/pristine prairies. Slight positive skew.

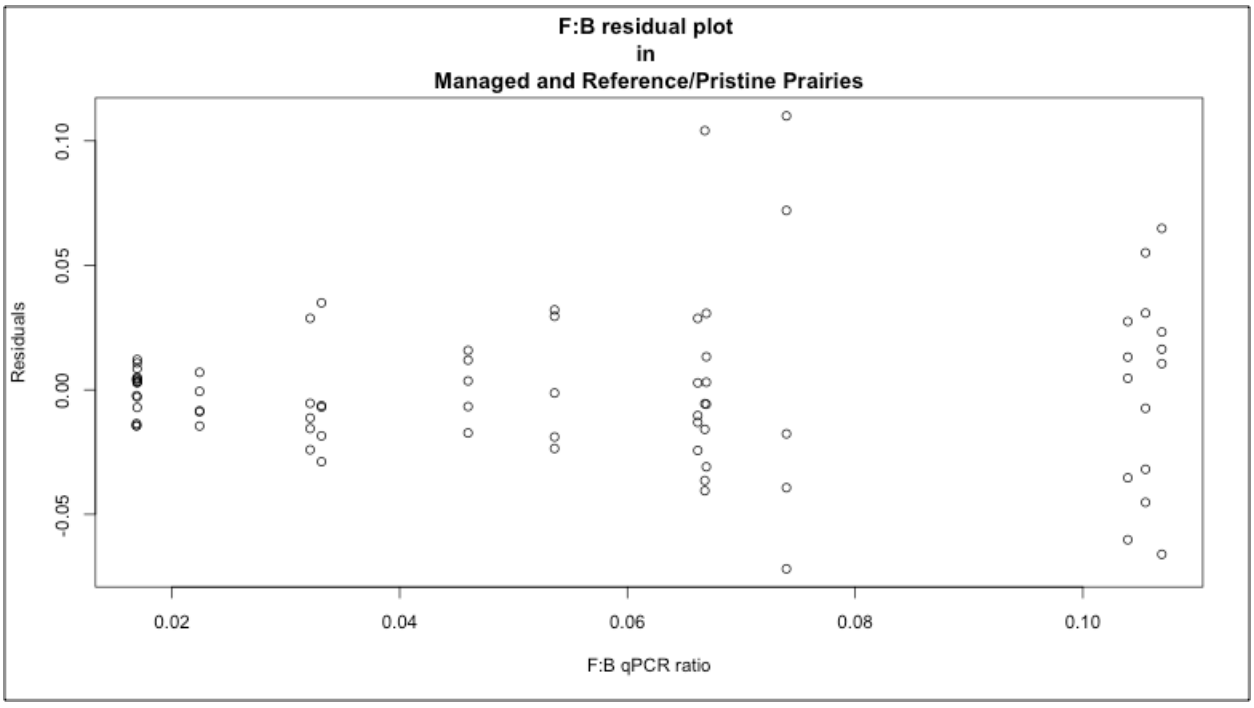

Figure 64. Residual plot of F:B gene copy ratios in Managed and Reference/Pristine Prairies.

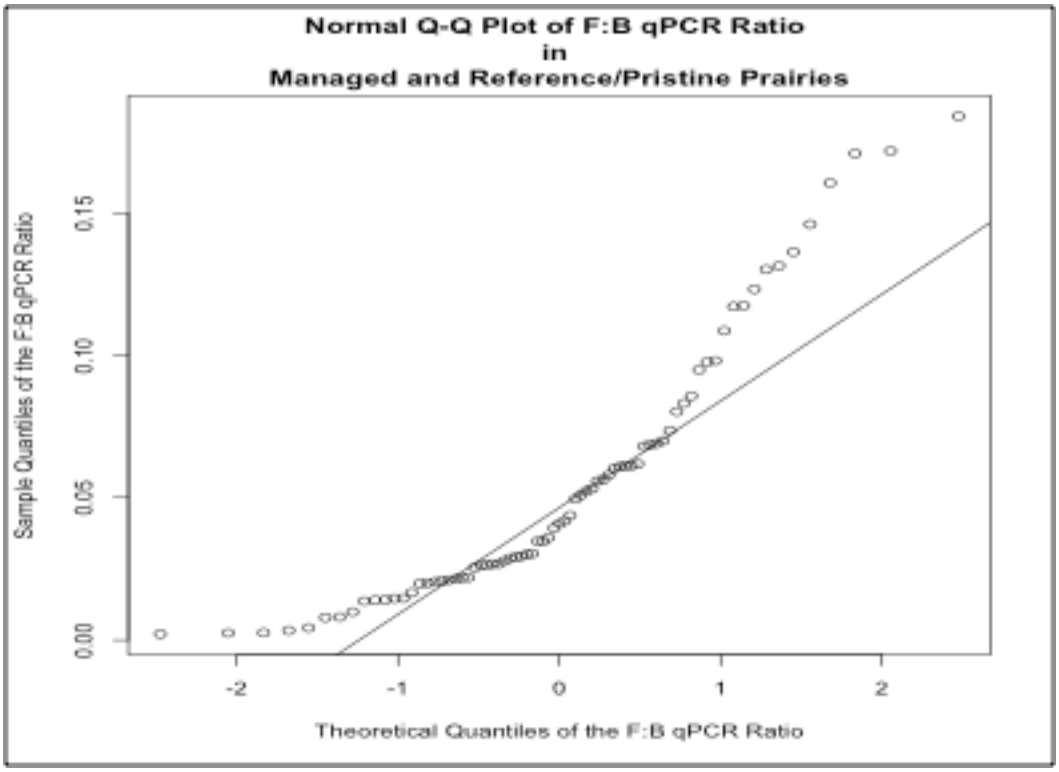

Figure 65. QQ Plot of F:B qPCR Ratio in Managed and Reference/Pristine Prairies. Deviates from normal.

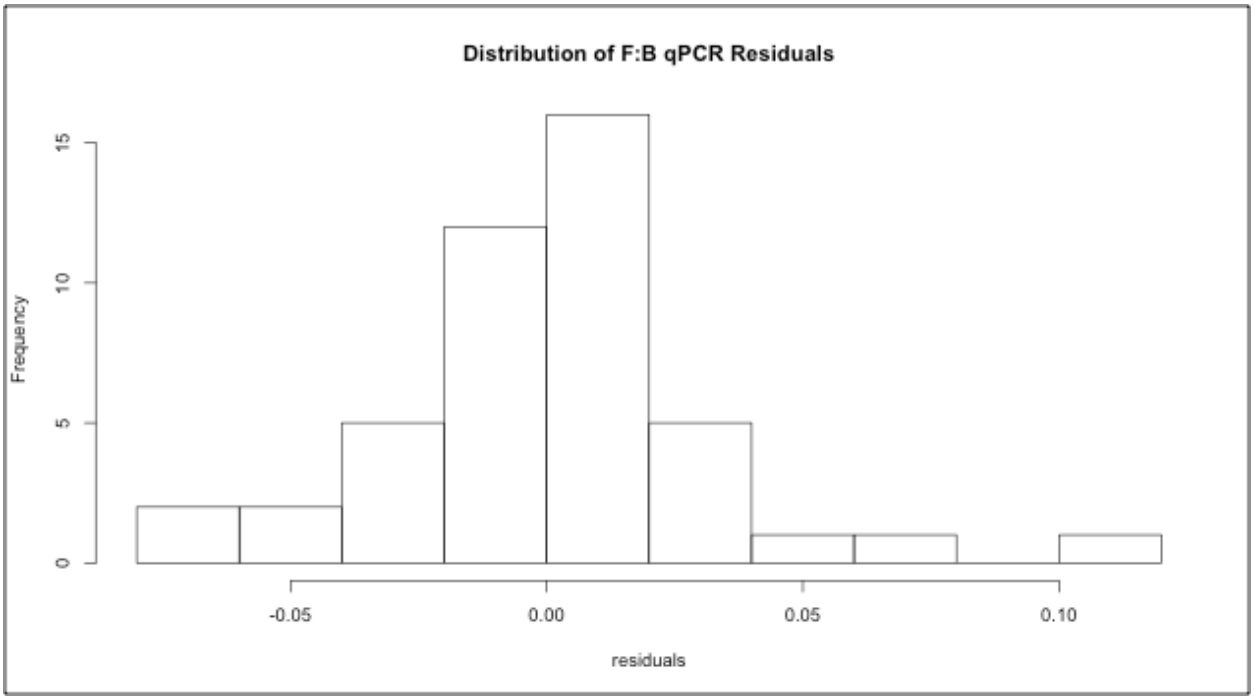

Figure 66. Histogram of F:B qPCR residuals in management sequence. Slight positive skew.

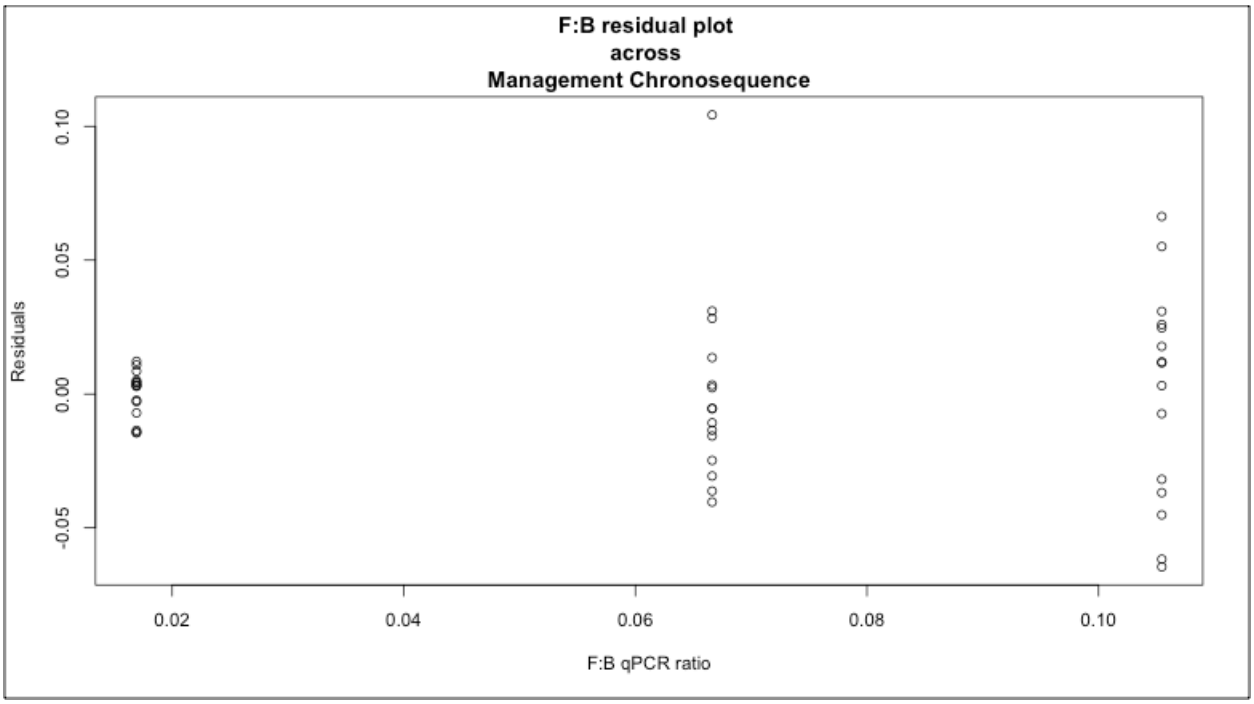

Figure 67. F:B residual plot across management chronosequence.

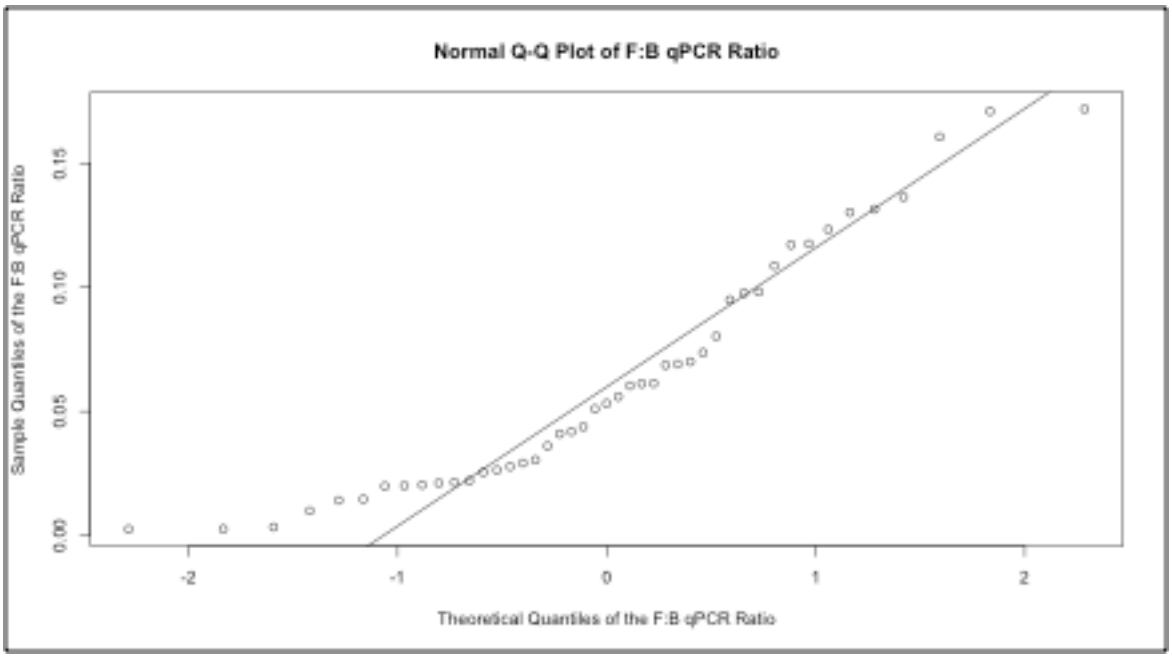

Figure 68. Q-Q Plot for F:B across management chronosequence. Not major deviation from normal.

Table 6. Known Sites Histories.

Site Name	Management Category	Catogory Code	County	Year Land Preserved	Year Restoration Began	Previous Land Use	Restoration techniques employed	Burned	Plowed	Seeded	Herbicide	Tiles removed
Hawk Hollow Bartlett Road Meadow	Unmanaged	R0	DuPage	1972-2010	na	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Hawk Hollow Northwest	Unmanaged	R0	DuPage	1972-2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Hawk Hollow Northeast	Unmanaged	R0	DuPage	1972-2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
SBP Central Prairie	Early-stage management	R1	DuPage	1974-2005	n/a	agriculture, manipulated part of the creek for ag	n/a	n/a	n/a	n/a	n/a	n/a
Smith Road Prairie	Early-stage management	R1	DuPage	1965, 1974-2007	n/a	Agriculture; lake and 3 ponds dug as gravel pits when land was privately owned.	n/a	n/a	n/a	n/a	n/a	yes
Bergman	Early-stage management	R1	Cook	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
SBP South Fields	Prolonged management	R2	DuPage	1974-2005	n/a	agriculture, manipulated part of the creek for ag	na	na	na	na	na	na
Half Day	Prolonged management	R2	Lake	n/a	n/a	na	na	na	na	na	na	na
Cuba Marsh	Prolonged management	R2	Lake	n/a	n/a	na	n/a	n/a	n/a	n/a	n/a	n/a
Grant Woods	Reference restoration	R3	Lake	n/a	n/a	Occupied by Potawatomi until 1830s, then homesteaded and farmed by different families.	n/a	n/a	n/a	n/a	n/a	n/a
Glacial Park Pioneer Road South	Reference restoration	R3	McHenry	n/a	n/a	Farmed by Weidrich family from 1875 to 1975	n/a	yes	n/a	n/a	yes	yes
Glacial Park Pioneer Road North	Reference restoration	R3	McHenry	n/a	n/a	Farmed by Weidrich family from 1875 to 1975	n/a	yes	n/a	n/a	yes	yes
Somme Prairie	Pristine remnant	P3	Cook	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Wadsworth Prairie	Pristine remnant	P3	Lake	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Larson Prairie	Pristine remnant	P3	McHenry	n/a	n/a	n/a	n/a	na	n/a	n/a	n/a	n/a

Table 7. Biological soil property data.

Full appendix may be available upon request. For more information, contact:
Dr. Louise Egerton-Warburton (lwarburton@chicagobotanic.org)

Table 8. Mean soil texture and phosphorous by site. Data collected in July 2012.

Full appendix may be available upon request. For more information, contact:
Dr. Louise Egerton-Warburton (lwarburton@chicagobotanic.org)

Table 9. Physical soil property data.

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Table 10. Chemical soil property data.

Full appendix may be available upon request. For more information, contact:
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