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Belowground Ecosystem Recovery During Grassland Restoration: South African Highveld Compared to US Tallgrass Prairie

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Abstract

Conversion of cultivated land to grassland is globally practiced to reverse soil degradation, but belowground ecosystem response to restoration has never been compared between old and new world temperate grasslands. We used a chronosequence approach to model change in root biomass and quality (indexed by C:N ratio), microbial biomass and composition [indexed by phospholipid fatty acids (PLFAs)], soil aggregate structure, and soil C and N stocks in the South African Highveld and compared recovery of these variables to a grassland restoration chronosequence in the US tallgrass prairie. We hypothesized soil C recovery, and mechanisms promoting soil C and N accrual would be convergent between these distant temperate grasslands with similar growing season precipitation, history of cultivation, and undergoing restoration with C_4-grasses. Total PLFA richness and concentrations of most microbial groups rose to represent uncultivated grassland in the highveld (similar to tallgrass prairie), but in contrast to tallgrass prairie, the fungi:bacteria ratio did not increase with restoration age. In the highveld, root biomass accumulation was lower, but root quality became more representative of the never-cultivated grassland than in restorations in tallgrass prairie. Soil aggregate recovery was slightly faster in tallgrass prairie, and the pattern of macroaggregate C recovery was divergent due to less depletion in cultivated soil and higher stock of C in the uncultivated soil relative to the highveld. More rapid restoration of total soil C and N stocks in the highveld was attributed to greater soil C saturation deficit at the onset of restoration, development of higher quality root systems that promote the microbial biomass and soil aggregation, and climate conditions (distinct periodicity of rainfall and high aridity) that likely impose more limitation to decomposition relative to the tallgrass prairie ecosystem.

Key words: aggregate; carbon; microbial community; nitrogen; phospholipid fatty acid; root; soil.

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Introduction

Grassland is considered an ''anthropogenic biome'' due to long-term human influence for the production of crops and livestock (Ellis and Ramankutty 2008). Conversion of grassland to conventionally tilled agricultural systems leads to increased erosion, depletion of organic matter stocks, less nutrient conservation, and reduced water infiltration (Haas and others 1957; Low 1972; Mann 1986). Restoring cultivated land to perennial grasses improves many ecosystem services provided by grasslands (Baer and others 2012). Space-for-time substitutions or chronosequence experimental designs have been used to quantify the rate of soil recovery in response to grassland restoration and predict time required for soil conditions to approach steady state in the US tallgrass prairie (Baer and others 2002; McLauchlan and others 2006; Matamala and others 2008; Baer and others 2010) and the South African highveld (Preger and others 2010; Lauer and others 2011; Koesters and others 2013). No studies have directly compared belowground recovery in response to grassland restoration between these regions that both contain C_4-dominated grassland, cropland, and formerly cultivated soil planted to C_4-grasses.

Cultivation of grassland lowers total C and nitrogen (N) stocks to a new equilibrium (Parton and others 1988), but the decay rate of C and N varies with climate, soil depth, and mineral fraction. The impact of cultivation and recovery of soil structure and function through restoration is generally ascertained using grassland that has never been cultivated as a reference for steady-state or historic (*sensu* original) condition. Parton and others (1988) estimated a 23–25% loss in soil organic C and N in the surface 20 cm of soil following 60 years of cultivation in the Great Plains of North America, but up to 70% loss has been documented (Mann 1986). Rapid exponential decay rates of soil C and N have also been documented in the highveld region of South Africa, where after 30–35 years of cultivation, soil C and N in the 0–20 cm depth approached a new equilibrium that contained 75 and 73% less soil organic C and total N, respectively, relative to uncultivated grassland (du Toit and others 1994).

The conversion of conventionally cultivated systems to perennial grassland has been advocated as a means to conserve soil and mitigate climate change (Conant and others 2001; Follett and others 2001; Lal and others 2011). Sequestration of C in soil depends on factors influencing the stabilization of organic matter, i.e., chemical protection through mineral sorption (Sørenson 1972), physical protection in aggregates (Tisdall and Oades 1982; Elliott 1986; Six and others 2000), and biochemical protection through recalcitrance (Krull and others 2003). Sowing perennial prairie grasses into former cropland in the US increases the quantity of low-quality (less decomposable) root biomass over time (Baer and others 2010), which should promote C inputs and aggregation of soil through root (and hyphae) entanglement and microbial turnover (Oades and Waters 1991). Indeed, multiple studies have demonstrated that the soil microbial biomass and the ratio of fungi:bacteria increase in response to grassland restoration in the tallgrass prairie ecosystem (Allison and others 2005; Matamala and others 2008; Bach and others 2010), and these microbial community changes coincide with C accumulation in large soil aggregates (Baer and others 2010; O'Brien and Jastrow 2013).

The overall objective of this study was to investigate whether patterns in belowground recovery from cultivation through grassland restoration in the tallgrass prairie ecosystem are convergent with formerly cultivated old-world grasslands established with functionally similar species (C_4 grasses) under comparable growing season precipitation. We quantified belowground ecosystem properties across a chronosequence of grassland restorations in the Free State of South Africa and compared recovery patterns to a grassland restoration chronosequence located in Nebraska, USA (Baer and others 2010). The chronosequence in South Africa we studied has been used to quantify changes in soil organic matter and C stocks in clay, silt, and sand fractions of soil (Preger and others 2010; Koesters and others 2013). The novel intent of this study was to model the relationship between restoration and belowground biological and structural properties to provide a better understanding of mechanisms responsible for C and N accrual in whole soil and aggregate fractions. The comparability of soil properties measured between the continents was made possible by using the same sampling, processing, and analytical methods as Baer and others (2010). There was, however, no way to sample taxonomically similar soils due to the different geologic and glacial histories of the two regions. We selected grassland plantings in South Africa established on soil with the most similar clay content to restorations studied in Nebraska. The plantings in South Africa were in close proximity to grassland that had never been cultivated and contained stocks of C and N similar to

tallgrass prairie in Nebraska. We predicted similar patterns (convergence) in many aspects of belowground restoration in response to establishing C_4-grasses on formerly cultivated land in South Africa and North America due to similar regional grassland productivity, precipitation received during the growing season, clay content of soil, and functionally similar plant communities. We also hypothesized that the mechanisms promoting C accrual (developing low-quality root systems, increasing fungi:bacteria ratio of the microbial biomass, and improved aggregate structure) in the South African Highveld would parallel those in the tallgrass prairie. Because most studies that have modeled belowground ecosystem recovery in response to grassland restoration have been conducted in tallgrass prairie (with much less weathered soil relative to many other grasslands, globally), we postulated that this regional comparison would reveal generalizations or new insights into factors influencing C accrual in developing grassland, which could be used to obtain better estimates of C sequestration potential of agricultural land (Conant and others 2001; Lal and others 2011).

Methods

Study Sites

The study sites in South Africa were located near Harrismith in the Free State Province (Appendix A). The region is characterized as Moist Cool Highveld Grassland, also known as *Cymbopogon-Themeda* Veld and *Themeda triandra-Eragrostis curvula* Grassland (Low and Rebelo 1996). The grasslands are commonly grazed with an average stocking density of 0.4 large stock units ha^{-1} (Lobe and others 2002). The regional climate has been characterized as receiving 635 mm of rainfall annually and in a discrete period from October to March (growing season). The mean annual temperature has been 13.8°C (Lobe and others 2001). Soils were characterized as fine loamy sand to fine sandy loam, thermic, mixed Typic Plinthustalfs with less than 5% slope.

The South Africa chronosequence consisted of four cultivated fields in fallow rotation, 17 restored grasslands ranging from 4 to 44 years since sowing, and three grasslands (velds) that had never been cultivated (Appendix A). Cropping systems in the region include rotation of fallow, wheat (*Triticum aestivum*), maize (*Zea mays*), and to a lesser extent sunflower (*Helianthus* sp.) (Lobe and others 2005). All but one of the restored grasslands was sown with the long-live perennial grass, *Eragrostis curvula* (Schrad.) (Nees); the exception was sown with *Digitaria smutssi* [Stent]. Native grasses colonize naturally. Management of sown grasslands consisted of burning approximately every 5 years and applying inorganic N and P fertilizer to promote grass establishment (Preger and others 2010). Percent loss of the soil organic C concentration was shown to decline exponentially to equilibrium following 34 years of cultivation (du Toit and others 1994; Lobe and others 2002). All sites were cultivated for at least 20 years prior to grassland restoration. Thus, C stocks should have approached this equilibrium.

The chronosequence in South Africa was compared to that on soil with similar clay content, history of cultivation, and restored with C_4-grasses in southeast Nebraska, US. Soils of study sites in Nebraska were classified as silty clay loam (Fine smectitic, mesic Aquertic Argiudolls) formed by loess with 0–6% slope. The Nebraska chronosequence consisted of 25 fields (including three crop fields) restored from 0 to 19 years and three prairies that had never been cultivated. During the restoration period (1988 through 2008), average annual precipitation in the region (Beatrice, Nebraska Weather Station; 40.2994 N, 96.75 W) was 757 mm, of which an average of 616 mm was received during the growing season (April through September). Average annual, minimum, and maximum temperatures during this period were 10.9, 4.2, and 17.6°C, respectively. All restored grasslands in Nebraska were sown with native perennial C_4-grasses. Soil texture and species planted at each site are described in Baer and others (2010).

Field Sampling

Methods used to determine C and N in roots, soil microbial biomass, and soil aggregate fractions in this study followed those used by Baer and others (2010) and Bach and others (2010), with the exception that the 0–10-cm depth was sampled in Nebraska, and the 0–5- and 5–10-cm depths were sampled in South Africa. We separated soil cores into the 0–5- and 5–10-cm depths in South Africa because we were initially uncertain whether the 0–10-cm depth would capture soil changes if they occurred only in a very shallow depth.

We sampled soil using two different methods in each field. Approximately 20 soil cores (2-cm dia.) were randomly taken from each field (collected several meters apart from one another to encompass a representative sample from the whole field)

and composited to quantify microbial community composition and soil C and N stocks. Each soil core was separated into the 0–5- and 5–10-cm depths, then composited by site and depth. We also removed eight intact 5.5-cm dia. soil cores to quantify root biomass, aggregate fractions and associated C and N, soil mass, and soil texture for the 0–5- and 5–10-cm depths at each site. These intact soil cores were taken at least 10 m apart and across an area that represented the whole field. Upon removal from the field, each intact core was placed in a polyethylene bag, inserted into an aluminum sleeve to prevent compaction, and stored at 4°C.

Belowground Ecosystem Properties

Immediately following field sampling, the composite soil samples were homogenized through a 4-mm mesh sieve. Two 30-g subsamples from each field and depth were immediately frozen (−20°C). Frozen subsamples were shipped to the Soil Microbial Ecology Laboratory (University of California, Davis) for phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from soil using the chloroform extraction method developed by Bligh and Dyer (1959) and modified according to Bossio and others (1998) for this purpose (DeGrood and others 2005) and described by Bach and others (2010). PLFAs were identified and quantified using standards and a library from Microbial Identification System (Microbial ID, Inc., Newark, DE). PLFAs were assigned to microbial groups according to Bach and others (2010) (Appendix B). The total number of unique, non-plant fatty acids was used as an index of total PLFA richness. This PLFA index, however, does not represent microbial taxonomic richness or diversity (Frostegård and others 2011), but changes in the index across the chronosequence indicate changes in microbial cell composition resulting from microbial community and/or physiological change as grassland develops.

Each intact soil core was weighed, carefully crumbled along natural planes of weakness to conserve the soil aggregate structure, and passed through an 8-mm mesh sieve. Large root fragments were separated from the soil, and a 100 g subsample of soil was intensively picked to retrieve fine roots. Roots from the 0–5- and 5–10-cm depths of each core were combined and washed with deionized water over a 150-μm mesh sieve. No attempt was made to distinguish between live and dead roots. Roots were dried at 55°C, weighed, ground, and re-dried. Percent C and N in root tissue were determined on a Thermo Flash 1112 CN Analyzer (distributed by CE Elantech Corp., Lakewood, New Jersey, USA).

Soil bulk density was calculated from the moisture content of each intact core fresh weight. Two 50-g subsamples were removed from each soil core processed for roots to determine gravimetric water content. We used the average minimum mass of soil from the three sites with the lowest bulk density to express all soil C and N stocks on an equivalent soil mass basis (Lee and others 2009). Due to the positive relationship between soil bulk density and depth and the inverse relationship between soil C and N storage with depth, the minimum mass represents a conservative estimate of C and N stocks because sites with higher bulk density include more soil from the deeper depth that would otherwise have been excluded if it were possible to sample sites by the same mass. The average minimum masses of soil in the 0–5- and 5–10-cm depths were 67.78 and 55.76 kg m^{-2}, respectively.

After roots and subsamples for gravimetric water content were removed from each intact soil core, the 8 cores from each site were combined and homogenized by site and soil depth. Three 100 g subsamples were removed and air-dried for aggregate isolation. Large macroaggregates (>2,000 μm), small macroaggregates (2,000-250 μm), free microaggregates (250-53 μm), and silt and clay (<53 μm) were isolated from one of the 100 g subsamples by wet-sieving according to Six and others (2000). Each fraction was dried at 105°C and weighed. Dry aggregate masses were used to determine aggregate size distribution and calculate the mean weighted diameter (MWD) of the soil aggregates in each field using the following equation: MWD (mm) = 5 × (P_w > 2000 μm) + 1.125 × (P_w > 250 μm) + 0.157 × (P_w > 53 μm) + 0.027 × (P_w < 53 μm), where P_w was the proportion of total dry mass for each aggregate size class.

Carbon and N stocks were quantified for the whole soil (from the composite samples) and each aggregate fraction. Replicate subsamples of soil were dried at 55°C, ground to a fine powder, and 50–70 mg of soil was dry combusted to determine %C and %N on a Thermo Flash 1112 CN Analyzer. Total C and N stock was calculated by summing the stock of C and N in the 0–5-cm depth and the stock C and N in the 5–10 cm minus the stock in the mass of soil in excess of the minimum equivalent mass.

Following removal of subsamples from the combined intact soil cores for aggregates, a 100 g subsample of soil was air-dried and shipped to the

University of California Soil Testing Lab (Davis, California) to determine percent sand, silt, and clay with the pipette method.

Statistical Analyses

We used Table Curve 2D 5.01 (SYSTAT Software Inc. 2002) to initially assess which simple model best described changes in response variables within the chronosequence of restored grasslands in South Africa using model rankings generated by coefficients of determination and significance level of the model fit. Following model selection, curve fitting was performed using Sigma Plot (SYSTAT Software Inc. 2008).

We modeled recovery of belowground ecosystem properties between the two regions using linear and exponential models with the never cultivated grassland soil assigned an age of 1,000 years (Matamala and others 2008; Baer and others 2010; O'Brien and Jastrow 2013). All variables significantly fit three-parameter exponential rise to maximum or exponential decline models, with the exception of root tissue C:N ratio in Nebraska, which increased linearly across the chronosequence to exceed uncultivated tallgrass prairie; hence, the prairies were not included in the model. If an exponential rise to maximum model significantly fits the response variable in both regions, we used the non-linear (NLIN) model procedure to compare trends between groups (SAS/STAT(R) 2014). We contrasted the residual sums of squares (SS) between a reduced (red) model using data from both regions (1 set of parameter estimates) with a full (f) model in which the parameters were varied by each region. The F statistic for the sums of squares reduction test ('hypothesis of equal pattern') was calculated accordingly:

$$F_R = ((SS_{red} - SS_f)/(df_{red} - df_f))/ms_{red},$$

where ms = mean squared error and df = degrees of freedom. The corresponding P value was generated from the following equation: $1 - \text{Probf}(F_R, df_{red} - df_f, df_f)$.

To predict time for each variable fit to an exponential model to reach steady state, we defined equilibrium (S) as 99.9% of uncultivated soil C and N stocks:

$$0.999S = S_o + (S_e - S_o)\left(1 - e^{-kt}\right),$$

where S_o is the parameter estimate at the onset of restoration or lower equilibrium resulting from long-term cultivation, S_e is the parameter estimate of steady-state condition, k is the rate constant, and t is the time. Using this equation, we then solved for time:

$$t = \text{Log}[0.001S_e/(S_e - S_o)] \times (-1/k).$$

Results

Belowground Changes During Grassland Restoration in the South Africa Highveld

Total PLFA richness and biomass of many microbial groups in the surface 5-cm of soil increased across the restoration chronosequence to represent the never-cultivated highveld (Figure 1), but not in the 5–10 cm soil depth increment (data not presented). The number of unique PLFAs (richness), total PLFA biomass, and concentrations of Gram-positive bacteria, Gram-negative bacteria, actinomycetes, arbuscular mycorrhizae fungi (AMF) indicative, and total fungal PLFAs approached the uncultivated highveld following approximately 25 years of C_4 grass establishment (Figure 1). These same groups increased with restoration across the Nebraska chronosequence but required 44 years to reach native prairie levels (Bach and others 2010). In contrast to Nebraska, saprotrophic fungi (Figure 1G) and the fungi:bacteria ratio (data not presented) did not change across the restoration chronosequence in South Africa. We assigned three PLFAs to saprotrophic fungi (18:3ω6, 18:0 anteiso, 18:2ω6,9) to be consistent with other studies that have examined changes in this group during grassland restoration (Allison and others 2005; McKinley and others 2005; Allison and others 2007), but Frostegård and others (2011) suggest only18:2ω6,9 is a good indicator of this group.

The proportional distribution of aggregate fractions became representative of uncultivated grassland following nearly a half century of restoration in South Africa (Figure 2). The proportion of macroaggregates (large + small) in the 0–10-cm depth followed an exponential rise to maximum across the restoration chronosequence (Figure 2A). Both the proportion of microaggregates and silt and clay fractions declined exponentially across the chronosequence (Figure 2B, C). The proportional increase in macroaggregates and corresponding decrease in the smaller aggregate fractions resulted in an exponential rise in MWD to represent the uncultivated highveld within three decades of grassland establishment (Figure 2D).

The proportions of C and N distributed among macroaggregates (large and small combined), free microaggregates, and silt/clay fractions across the restoration chronosequence became representative

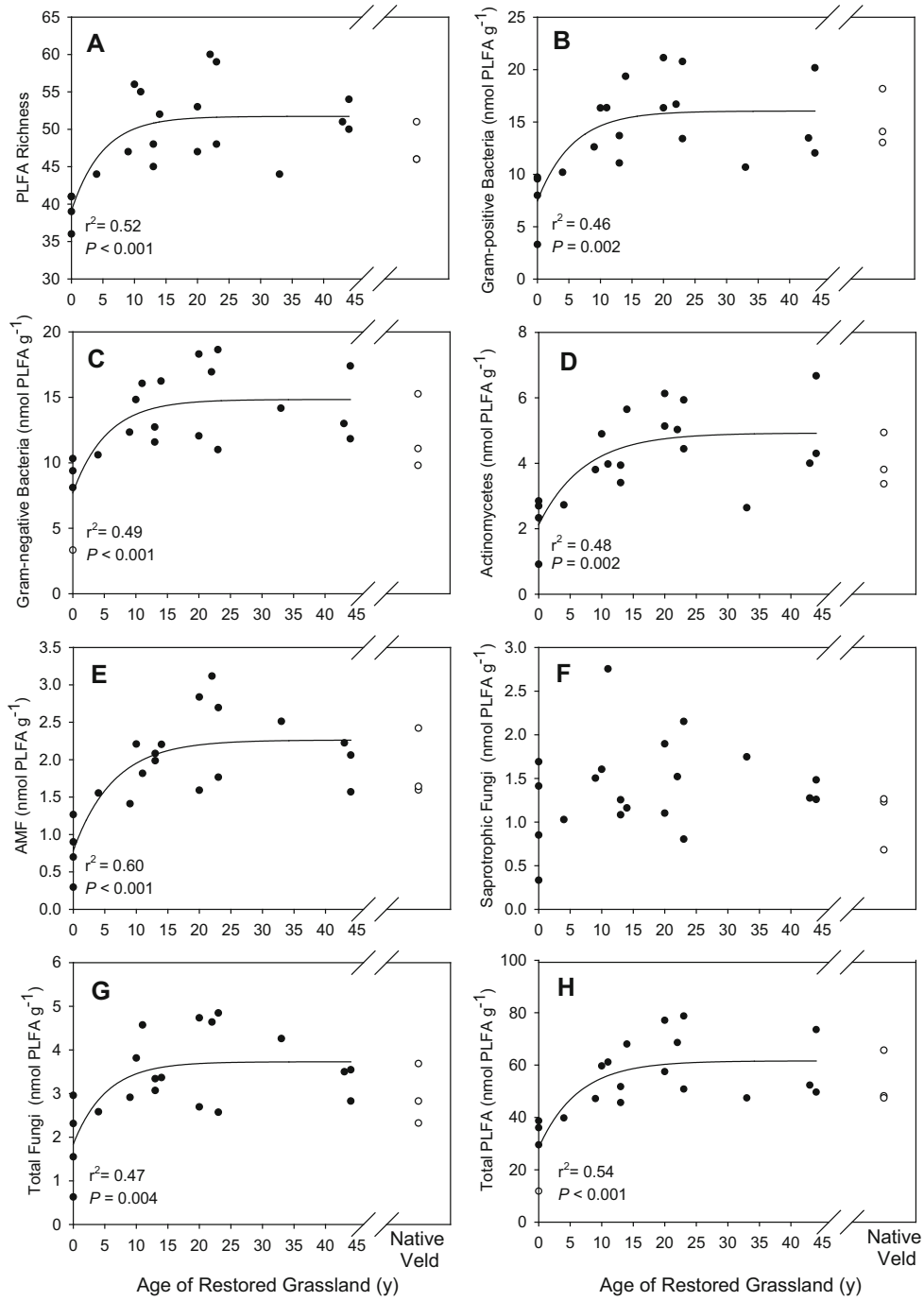

Figure 1. Change in **A** soil microbial richness as indexed by the number of unique phospholipid fatty acids [PLFAs], **B** Gram-positive PLFA biomass, **C** Gram-negative PLFA biomass, **D** actinomycete PLFA biomass, **E** arbuscular mycorrhizae [AMF] biomass indicative PLFA, **F** saprotrophic fungal PLFA biomass, **G** total fungal PLFA biomass, and **H** total PLFA biomass in the 0–5-cm depth across the restoration chronosequence and soil that had never been cultivated in the South Africa highveld. All PLFA response variables were fit to exponential rise maximum models [richness = $39.1 + 12.6(1 - e^{-0.202x})$; Gram-positive bacteria = $7.5 + 8.6(1 - e^{-0.180x})$; Gram-negative bacteria = $7.7 + 7.1(1 - e^{-0.184x})$; actinomycetes = $2.1 + 2.8(1 - e^{-0.139x})$; AMF = $0.8 + 1.5(1 - e^{-0.156x})$; total fungi = $1.8 + 1.9(1 - e^{-0.192x})$; and total biomass = $28.4 + 33.1(1 - e^{-0.161x})$]. Native velds were not included in the models.

of the uncultivated highveld within 50 years of grassland restoration (Figure 3). We combined the large and small macroaggregate fractions to clearly present changes among the three major aggregate fractions across the chronosequence. The proportions of C and N in the combined macroaggregate

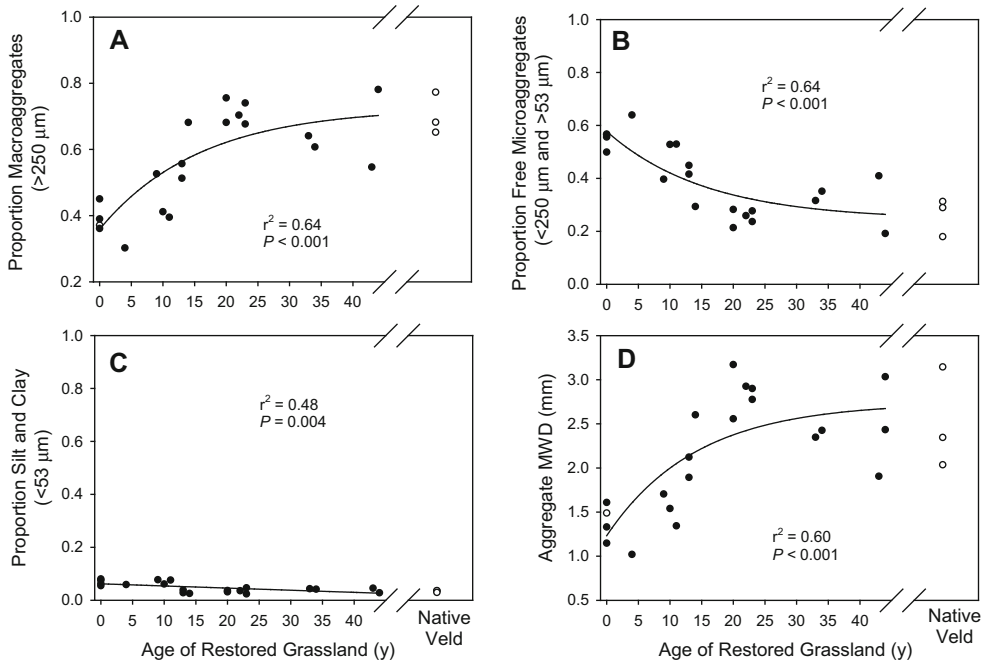

Figure 2. The proportion of **A** macroaggregates, **B** free microaggregates, **C** and silt and clay, and **D** aggregate mean weighted diameter in the 0–10-cm depth of soil across the chronosequence of restored grasslands and grassland that had never been cultivated in the South Africa highveld. Proportions of macroaggregates and aggregate MWD were fit to exponential rise maximum models [proportion of macroaggregates = $0.36 + 0.37(1 - e^{-0.063x})$; MWD = $1.23 + 1.51(1 - e^{-0.07x})$]. Free microaggregate and silt and clay proportions declined exponentially (proportion of free microaggregates = $0.24 + 0.33e^{-0.063x}$; proportion of silt and clay = $0.03 + 0.04e^{-0.077x}$]. Native velds were not included in the models.

fraction rose exponentially, whereas the proportion of C and N in the free microaggregate and silt and clay fractions declined exponentially across the restoration chronosequence (Figure 3A, B).

Comparison of Modeled Recovery to Uncultivated Highveld and Tallgrass Prairie

Belowground biomass and root tissue quality exhibited divergent patterns in Nebraska and South Africa (Table 1; Figure 4). The oldest restored grasslands in Nebraska contained nearly twice as much root biomass (339 ± 35 g m^{-2}) in the 0–10-cm depth and 41% higher C:N ratio of roots (79.1 ± 4.0) in the 0–20-cm depth than the oldest restored grasslands in South Africa. In Nebraska, root biomass increased at a rapid rate and root C:N surpassed that of prairie in the 19 years chronosequence (Figure 4A, B). The early asymptote in the development of root biomass in South Africa resulted in more than twice the time predicted for recovery (Table 1), but after four decades of grassland establishment, root quality was similar to that of uncultivated highveld.

Uncultivated grasslands in Nebraska and South Africa contained similar aggregate MWD, but the actively cultivated soil in Nebraska had an approximately 35% smaller aggregate MWD relative to South Africa. There was moderately faster recovery of aggregate MWD across the Nebraska chronosequence (test of equal trends: $0.05 < P < 0.10$) resulting in a predicted recovery of soil structure in response to restoration approximately 20 years earlier in Nebraska than South Africa (Figure 4C).

Despite more rapid recovery of the aggregate structure in Nebraska, C accrual in restored grassland macroaggregate fractions, which contained the most C relative to the other aggregate fractions, was slower relative to South Africa (Table 1; Figure 4). A fairly similar recovery pattern of C in large macroaggregates in both regions but no recovery of C in small macroaggregates in Nebraska (data not presented), coupled with a higher steady-state stock of C in all macroaggregates, resulted in different recovery patterns for macroaggregate C stocks (test of equal trends: $P < 0.001$). There was a much longer (+82 years) time predicted for macroaggregate C to accumulate to 99% of steady state in Nebraska relative to South Africa (Figure 4D; Table 1). Despite a reduction in the pro-

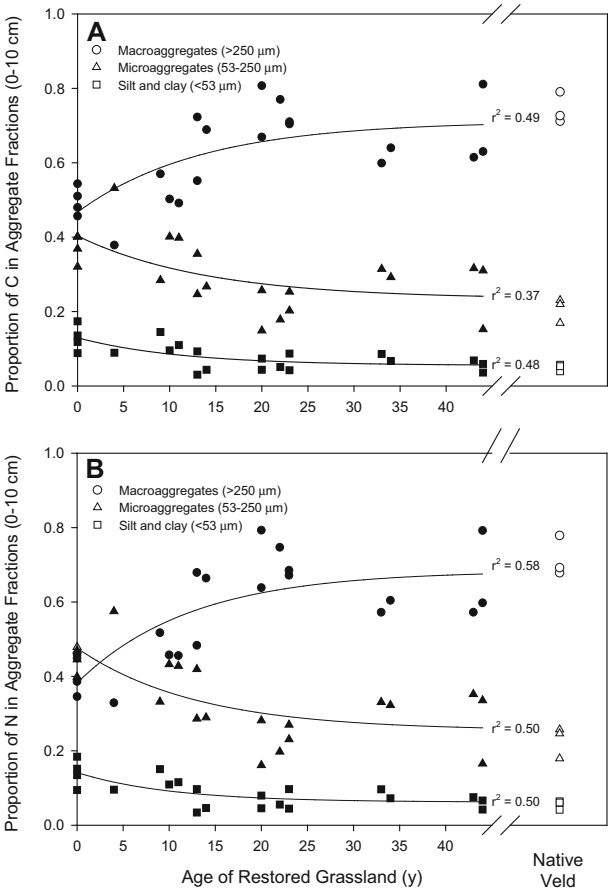

Figure 3. Change in the relative distribution of **A** carbon and **B** nitrogen among aggregate fractions in the 0–10-cm depth across the chronosequence of restored grasslands and in grassland that had never been cultivated in the South Africa highveld. Three-parameter power models best fit the change in the proportion of C [$y = 0.468 + 0.244 (1 - e^{-0.075x})$] and N [$y = (0.382 + 0.302(1 - e^{-0.081x})$] in the macroaggregate fraction. Three-parameter exponential decline models best described the change in the proportion of C in free microaggregate ($y = 0.234 + 0.169 \times e^{-0.071x}$) and silt/clay fractions ($y = 0.055 + 0.075 \times e^{-0.089x}$), as well as the proportion of N in the free microaggregate ($y = 0.255 + 0.222 \times e^{-0.077x}$) and silt/clay fractions ($y = 0.062 + 0.080 \times e^{-0.098x}$) in relation to age of restoration (x). All models were significant, $0.001 < P < 0.010$. Native velds were not included in the models.

portional mass of the free microaggregate fraction in South Africa and Nebraska (Baer and others 2010), there was no change in the C stock in free microaggregates across either chronosequence due to a higher concentration of C in this fraction as restoration proceeded (Figure 4E). Only in Nebraska was a change in C stock within the silt and clay fraction detected (Figure 4F), and the exponential decline model predicted C in this fraction to be at steady state in less than 5 years (Table 1).

The exponential rise to max models predicted recovery of total soil C (and N) in 42 (and 29) years in South Africa (Figure 4G and H; Table 1). There was, however, considerable variation in total C and N stocks in the surface 10 cm of soil among the three uncultivated highvelds included in the model. Two sites with the highest total C and N levels were actively grazed, whereas there was no evidence of grazing at the site with the lowest soil C and N. Excluding the ungrazed site resulted in more conservative estimates of 75 and 35 years for soil C ($y = 1260 + 1633 \times [1 - e^{-0.037x}]$; $r^2 = 0.053$, $P = 0.001$) and N ($y = 109 + 90 \times [1 - e^{-0.073x}]$; $r^2 = 0.043$, $P = 0.003$) to represent stocks in the uncultivated highveld.

Recovery patterns were moderately dissimilar for total soil C stock (test for equal trends: $0.05 < P < 0.10$) and highly dissimilar for total N stock (test of equal trends: $P < 0.001$) between the two regions (Figure 4G, H). Predicted recovery of the total C and N pools to steady-state conditions was approximately 100 years longer in Nebraska than South Africa (Table 1). This was due to a lower linear rate of C accrual in the first 2 decades (21.2 g C m^{-2} y^{-1} and 1.98 g N m^{-2} y^{-1}; Baer and others 2010) relative to South Africa (62.0 g C m^{-2} y^{-1}, $r^2 = 0.63$ and $P < 0.001$; 4.18 g N m^{-2} y^{-1}, $r^2 = 0.55$ $P < 0.001$) in the first 23 years of restoration. For total N, this was due to a lower linear rate of C accrual in the first two decades (21.2 g m^2 y; Baer and others 2010) relative to South Africa (62.0 g C m^{-2} y^{-1}; $r^2 = 0.36$, $P < 0.001$) in the first 23 years of restoration.

Discussion

This represents the only study to compare and contrast belowground ecosystem changes in response to developing C_4-perennial grassland between the new and old worlds. Contrary to our expectations, modeled recovery of soil C stock in the surface 10 cm in the highveld was predicted to occur in half the time or less (42–75 years) compared to tallgrass prairie (150 years). The shorter recovery rate estimated with the grazed restored grassland agrees with that determined by (Lauer and others 2011) using a subset of sites sampled in this study, 45.4 and 57.6 years in the 0–5 and 5–10 cm depths, respectively. The high accumulation rate of C during the initial decades of restoration was responsible for the more rapid recovery of soil C during grassland restoration in the highveld relative to tallgrass prairie. The high rate of soil C accrual during the first two decades of restoration in the highveld could be attributed to the lower

Table 1. Belowground Ecosystem Properties in Cultivated and Grassland Soil and Modeled Change in Response to Grassland Restoration in the South African Highveld (SA) and US Tallgrass Prairie (NEB)

	Site	Cultivated (observed)[1]	Grassland (observed)[1]	y_o (±SE)[2]	a (±SE)[2]	k (±SE)[2]	Model significance[3]	Time to 99% Grassland[4] (y)
Root biomass (g m^{-2})[5]	SA	20–31	355–382	69 ± 29	295 ± 51	0.014 ± 0.006	r^2 = 0.61***	203
	NEB[1]	28–52	460–806	58 ± 62	572 ± 90	0.040 ± 0.012	r^2 = 0.68***	74
Root C:N[6]	SA	22–26	58–68	26 ± 4	32 ± 5	0.082 ± 0.031	r^2 = 0.65***	34
	NEB[1]	45–36	46–56	44 ± 6	1.9 ± 0.4	–	r^2 = 0.48***	3
Aggregate MWD (mm)	SA	1.29–1.49	2.18–2.84	1.23 ± 0.22	1.40 ± 0.28	0.082 ± 0.038	r^2 = 0.56***	33
	NEB	0.90–0.92	2.23–2.79	0.89 ± 0.32	1.77 ± 0.34	0.247 ± 0.101	r^2 = 0.51***	12
Large + small macro C (g m^{-2})	SA	526–709	1746–1947	548 ± 239	1149 ± 324	0.057 ± 0.036	r^2 = 0.39**	50
	NEB	897–1049	2797–2878	1556 ± 81	1676 ± 141	0.021 ± 0.005	r^2 = 0.85***	132
Free micro C (g m^{-2})	SA	387–540	469–555	–	–	–	–	–
	NEB	406–599	431–520	–	–	–	–	–
Silt and clay C (g m^{-2})	SA	143–157	112–136	–	–	–	–	–
	NEB	211–243	109–128	98 ± 7	129 ± 20	0.883 ± 1.01	r^2 = 0.61***	3
Total C (g m^{-2})	SA	1072–1375	2422–3179	1171 ± 241	1391 ± 317	0.065 ± 0.034	r^2 = 0.49***	42
	NEB	1591–1817	3016–3190	1689 ± 67	1413 ± 118	0.018 ± 0.005	r^2 = 0.86***	149
Total N (g m^{-2})	SA	98–125	181–230	108 ± 18	83 ± 22	0.091 ± 0.058	r^2 = 0.41**	29
	NEB	164–178	274–290	161 ± 6	121 ± 11	0.020 ± 0.005	r^2 = 0.83***	134

[1]*Observed range of root biomass, root C:N, aggregate mean weighted diameter, and stock of C in each aggregate fraction and whole soil represent 1 standard deviation from the mean.*
[2]*Parameter estimates (±SE) are given for exponential rise to max ($y = y_o + a*[1 - e^{-kx}]$) and exponential decline ($y = y_o + ae^{-kx}$) models, where y_o = intercept and a = steady state–intercept.*
[3]*Variation explained and significance indicated by r^2 and asterisks, respectively (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).*
[4]*Time to 99% never cultivated grassland condition was calculated using significant models with steady-state conditions assigned to an age of 1,000 years.*
[5]*Root biomass data from Baer and others (2010) were reanalyzed for the 0–10-cm depth.*
[6]*C:N ratio of root tissue was measured in the 0–20-cm depth. A linear model ($y = y_o + ax$) best described change in root C:N ratio in Nebraska. Conditions did not approach native prairie, so native prairie was not included in the model.*

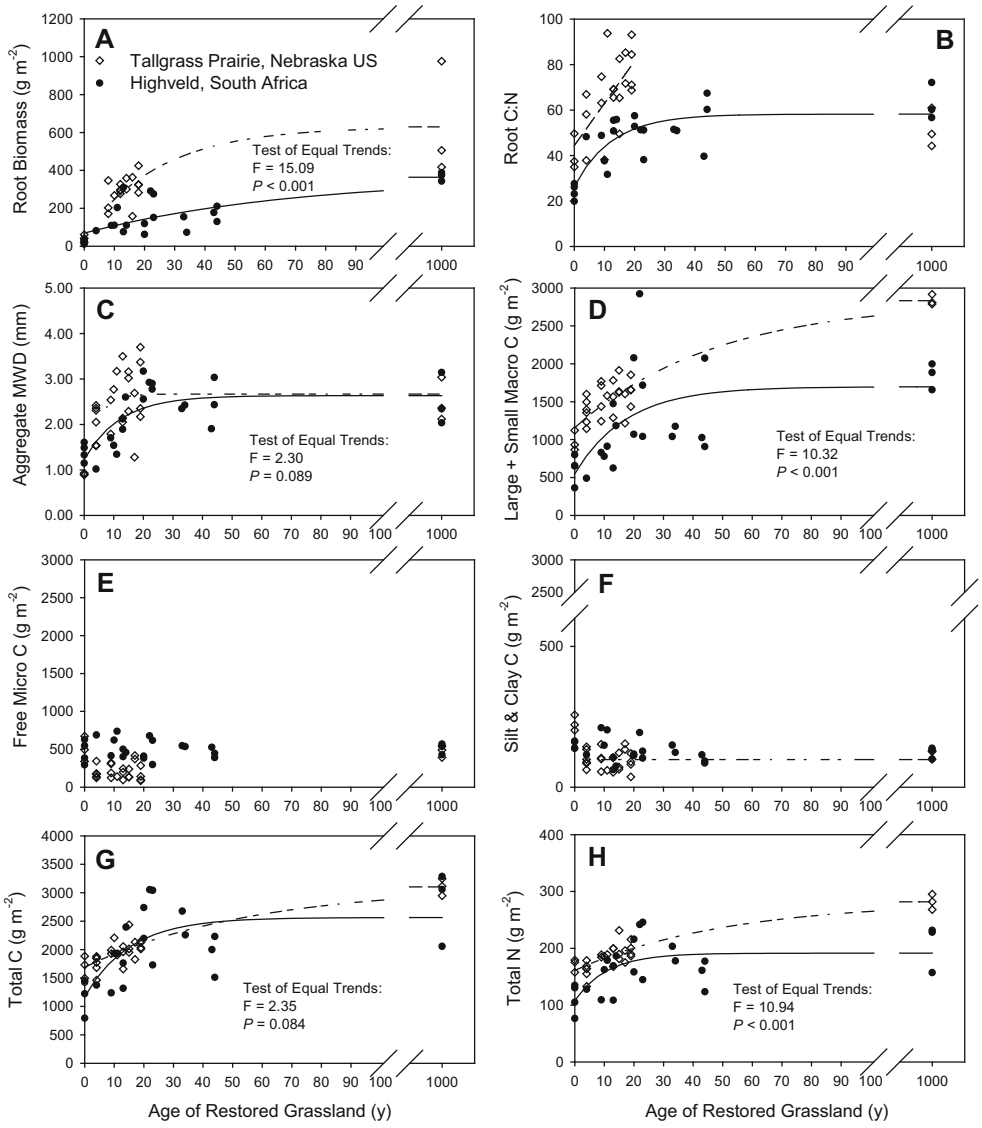

Figure 4. Modeled recovery of **A** root biomass, **B** root C:N ratio, **C** aggregate mean weighted diameter (MWD), **D** large + small macroaggregate C (>250-µm dia.), **E** free microaggregate C (micro; <250- and >53-µm dia.), **F** silt and clay C (<53-µm dia.), **G** total soil C, and **H** total soil N stock in US tallgrass prairie (Nebraska) and the South African Highveld. Lines fit to data indicate significant exponential models (Nebraska = *dashed*; South Africa = *solid*); corresponding predictive model parameter estimates are listed in Table 1. Differences in recovery patterns indicated by the *F* statistic and *P* value (that is, test of equal trends)

stock of C in the cultivated soil. Soil C is predicted to accumulate faster with more depletion relative to steady state or greater 'carbon saturation deficit' (Six and others 2002; West and Six 2007; Stewart and others 2007).

Nearly a quarter of sub-Saharan Africa contains land classified as 'severely degraded' due to agriculture, and restoring natural systems offers the most potential to improve soil and food security (Vågen and others 2005). Similar to Nebraska, the Free State Province of South Africa is extensively cultivated for grains. Cultivation has depleted the total soil C pool to a greater extent in the highveld (71% loss) relative to the tallgrass prairie (45% loss). The loss of soil C estimated in this study agrees closely with that reported by du Toit and others (1994) for the same region of South Africa. Greater depletion of soil C in the South African highveld likely occurred because of the coarser textured soil (Lobe and others 2001, 2002, 2005) and oxidation of less-protected C associated with the sand fraction. The tallgrass prairie soil contained 75% less sand, 293% more silt, and nearly an equivalent amount of clay relative to the highveld. Lobe and others (2001) attributed the low levels of silt in the highveld to wind erosion following cultivation of the primary grassland on less stable, coarsely textured soil.

Precipitation and soil texture influence soil C storage in cultivated and grassland soil but do not intuitively explain the variation in soil C recovery across the chronosequences of restored grassland in the tallgrass prairie and the highveld ecosystems. Sequestration of C in soil has been shown to increase with higher mean annual precipitation in agricultural systems (Paustian and others 1997),

and soil C storage increases from west to east in US grasslands corresponding to increasing precipitation (McCulley and others 2005). Mean annual precipitation in South Africa was 122 mm less than that received in the Nebraska study, but average growing season precipitation in Nebraska was highly similar to South Africa. Further, less C accrual would be anticipated in the coarser textured soil studied in South Africa, although (Preger and others 2010) documented more rapid but less complete recovery of C in the sand fraction in this soil. Rainfall periodicity and aridity, as they affect decomposition, could also partly be responsible for high soil C accumulation rates in South Africa relative to Nebraska. The highveld mostly only receives precipitation during the growing season, when temperatures are warmest. High evapotranspiration coupled with the more coarsely textured soils may supply ample water for plant growth but leave less favorable moisture conditions for decomposition in the growing season. Decomposition would further be limited during the extended period with negligible rainfall.

Biotic factors that could result in different recovery patterns of soil C (and N) include the rate at which root biomass develops, the quality of root inputs, root turnover, and soil microbial community structure. Recovery patterns of root biomass and root quality were distinct between the two regions. Root biomass and quality in the South Africa chronosequence became increasingly representative of the uncultivated highveld, whereas the biomass and C:N ratio of roots in the Nebraska chronosequence surpassed that of tallgrass prairie. The grasslands studied in Nebraska were predominantly restored with C_4 grasses and developed root systems of much lower quality (higher C:N ratio) than remnant prairie. Sampled prairies that were never cultivated in Nebraska contained more plant species and more C_3 forbs than the grass-dominated restoration (S. G. Baer, *personal observation*), which have a lower C:N ratio of root tissue (Craine and others 2002). Remnant prairies have also been shown to contain more fine roots relative to restorations (Kucharik and others 2006), which would further contribute to a lower C:N ratio of the root biomass. High inputs of low-quality roots not only should limit decomposition (Silver and Miya 2001; Krull and others 2003) but may also limit microbial growth and turnover that facilitate the protection of C through aggregate formation and stabilization through association with minerals (Six and others 2002). The higher quality inputs from roots during restoration in South Africa may also partly explain the faster recovery of the soil microbial communities and hence soil C (Gentile and others 2011).

Multiple studies have shown that restoration of tallgrass prairie on formerly cultivated soil increases saprotrophic fungi, AMF, Gram-positive bacteria, Gram-negative bacteria, and actinomycete PLFA concentrations (Allison and others 2005; McKinley and others 2005; Bach and others 2010). Despite the potential dilution effect associated with sampling deeper soil (Allison and others 2007), the remnant tallgrass prairie soil in Nebraska contained 83% more PLFA biomass (98.1 ± 4.3 nmol g^{-1}; Bach and others 2010) than the highveld grassland. The cultivated, oldest restored, and uncultivated prairie soil in Nebraska also had 54, 65%, and more than 200% greater concentrations of AMF-indicative 16:1 ω 5c PLFA than corresponding South Africa sites. The 16:1 ω 5c PLFA is commonly used to estimate AMF biomass as it has been shown to increase with increasing AMF colonization in plants, particularly in grasslands (Olsson 1999; Allison and Miller 2005). This PLFA can, however, be produced by some bacteria (Olsson 1999; Frostegård and others 2011); hence, concentrations of 16:1 ω 5c may not exclusively represent AMF biomass, further underscoring the importance of bacteria in South Africa grasslands. Bach and others (2010) predicted recovery of Gram-positive, Gram-negative, AMF, and total PLFA biomass within 44 years of grassland restoration on silty clay loam soil in Nebraska. All of these groups recovered well before 44 years of restoration in South Africa. In contrast to our expectation based on several studies in tallgrass prairie (Allison and others 2005; Matamala and others 2008; Bach and others 2010), the PLFA biomass ratio of fungi:bacteria did not increase across the South Africa chronosequence, as predicted in response to the cessation of cultivation (van der Wal and others 2006; Mahaming and others 2009) and in a coarse-textured soil (Sessitsch and others 2001). In fact, the PLFA biomass of bacteria increased proportionally more than fungi in the South Africa chronosequence, contradicting previous observations of microbial residues studied in this chronosequence (Lauer and others 2011). The fungal biomass may actually have contributed the most to organic matter accrual in South Africa through recalcitrance of the fungal necromass (Six and others 2006; Lauer and others 2011), but this group does not dominate the active microbial biomass measured by the PLFAs in the restored grassland or uncultivated highveld soil. This represents a distinct difference in recovery dynamics and natural

structure of the soil microbial community between these old and new world grassland systems.

There was divergence in recovery patterns of soil aggregate structure between the new- and old-world chronosequences, with highly dissimilar trends in the distribution of C and N among aggregate fractions. Aggregate structure in both regions was restored through the development of perennial root systems, although somewhat more rapidly in Nebraska. The exudates of roots and associated hyphae coupled with a growing microbial biomass, and turnover promotes incorporation of free microaggregates, silt, and clay into macroaggregates (Jastrow 1987; Oades and Waters 1991; Jastrow and others 1998; Matamala and others 2008). The distribution of C (and N) among the aggregate fractions and change across the restoration chronosequence in South Africa reflected changes in the mass of aggregate fractions, indicating that both structure and distribution of C and N are restored within 50 years of grassland establishment. Carbon is expected to reach equilibrium over time (Six and others 2002; Stewart and others 2007) and the proportion of C (and N) in the three aggregate fractions represented the never-cultivated grassland soil in South Africa within 50 years of grass establishment.

Differences in soil C saturation deficit and soil texture likely contribute to variation in C accrual rates during grassland restoration in the old and new worlds. Faster accumulation of soil C in South Africa relative to Nebraska could be attributed to a more depleted stock of C in South Africa. This suggests that organic matter may play a larger role in binding small macroaggregates in the more coarsely textured soil in South Africa. Interestingly, if sand content is very high ($>80\%$) and clay content is very low ($<10\%$), there is little recovery of soil C resulting from lack of soil microbial and aggregate development (no protection of C) in response to restoration (Baer and others 2010). Thus, a critical ratio of sand:clay or C:N ratio in roots might be needed for degraded soil to accrue C through physicochemical or biological mechanisms, respectively.

Conclusion

This is the first study to demonstrate that grassland establishment on former cropland ''restores'' (*sensu* original or extant state) total C and N stocks. Contrary to our predictions, the South Africa highveld and US tallgrass prairie ecosystems exhibited strongly dissimilar recovery patterns of root biomass, quality of roots, the soil microbial PLFA fungi:bacteria ratio groups, and C stock in macroaggregates as grassland restoration proceeded. Similar to tallgrass prairie, microbial PLFA richness, total PLFA biomass, mean weighted aggregate diameter, and proportion of C and N in aggregate fractions increased exponentially to a maximum across the chronosequence in the highveld region to represent uncultivated grassland, but the time frame for restoration of total soil C and N stocks to steady-state conditions in South Africa was shorter. We attribute the more rapid soil recovery from cultivation through grassland restoration to greater C saturation deficit at the onset of restoration, coupled with development of high-quality root systems promoting recovery of the microbial biomass and aggregate structure, and climate limitation to decomposition in the highveld grassland relative to the tallgrass prairie ecosystem.

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References

Allison VJ, Miller RM. 2005. Using fatty acids to quantify arbuscular mycorrhizal fungi. In: Podila G, Varma A, Eds. Basic research and applications. L.K. International Pvt. Ltd: New Delhi. p 141–61.

Allison VJ, Miller RM, Jastrow JD, Matamala R, Zak DR. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Sci Soc Am J 69:1412–21.

Allison VJ, Yermakov Z, Miller RM, Jastrow JD, Matamala R. 2007. Assessing soil microbial community composition across landscapes: do surface soils reveal patterns? Soil Sci Soc Am J 71:730–4.

Bach EM, Baer SG, Meyer CK, Six J. 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. Soil Biol Biochem 42:2182–91.

Baer SG, Kitchen DL, Blair JM, Rice CW. 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecol Appl 12:1688–701.

Baer SG, Meyer CK, Bach EM, Klopf RP, Six J. 2010. Contrasting ecosystem recovery on two soil textures: implications for carbon mitigation and grassland conservation. Ecosphere 1(1):Art. 5.

Baer SG, Heneghan L, Eviner V. 2012. Applying soil ecological knowledge to restore ecosystem services. In: Wall DH, Bardgett RD, Behan-Pelletier V, Herrick JE, Jones H, Ritz K, Six J, Strong DR, van der Putten WH, Eds. Soil ecology and ecosystem services. Oxford: Oxford University Press. p 377–93.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–17.

Bossio DA, Scow KM, Gunapala N, Graham KJ. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb Ecol 36:1–12.

Conant RT, Paustian K, Elliott ET. 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecol Appl 11:343–55.

Craine JM, Tilman D, Wedin D, Reich P, Tjoelker M, Knops J. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Funct Ecol 16:563–74.

DeGrood SH, Claassen VP, Scow KM. 2005. Microbial community composition on native and drastically disturbed serpentine soils. Soil Biol Biochem 37:1427–35.

du Toit ME, Du Preez CC, Hensley M, Bennie ATP. 1994. Effect of cultivation on the organic matter content of selected dryland soils in South Africa. South Afr J Plant Soil 11:71–9.

Elliott ET. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci Soc Am J 50:627–33.

Ellis EC, Ramankutty N. 2008. Putting people in the map: anthropogenic biomes of the world. Front Ecol Environ 6:439–47.

Follett RF, Pruessner EG, Samson-Liebig SE, Kimble JM, Waltman SW. 2001. Carbon sequestration under the conservation reserve program in the historic grassland soils of the United States of America. In: Lal R, McSweeney K, Eds. Soil carbon sequestration and the greenhouse effect. Madison: Soil Science Society of America. p 27–40.

Frostegård Å, Tunlid A, Bååth E. 2011. Use and misuse of PLFA measurements in soil. Soil Biol Biochem 43:1621–5.

Gentile R, Vanlauwe B, Chivenge P, Six J. 2011. Trade-offs between the short- and long-term effects of residue quality on soil C and N dynamics. Plant Soil 338:159–69.

Haas, HJ, Evans CE, Miles ER. 1957. Nitrogen and carbon changes in soils as influenced by cropping and soil treatments. Washington DC: USDA Technical Bulletin 1164. U.S. Government Printing Office.

Jastrow JD. 1987. Changes in soil aggregation associated with tallgrass prairie restoration. Am J Botany 74:1656–64.

Jastrow JD, Miller RM, Lussenhop J. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biol Biochem 30:905–16.

Koesters R, Preger AC, Du Preez CC, Amelung W. 2013. Re-aggregation dynamics of degraded cropland soils with prolonged secondary pasture management in the South African Highveld. Geoderma 192:173–81.

Krull ES, Baldock JA, Skjemstad JO. 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Funct Plant Biol 30:207–22.

Kucharik CJ, Fayram NJ, Cahill KN. 2006. A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. Global Change Biol 12:122–39.

Lal R, Delgado JA, Groffman PM, Millar N, Dell C, Rotz A. 2011. Management to mitigate and adapt to climate change. J Soil Water Conserv 66:276–85.

Lauer F, Koesters R, du Preez CC, Amelung W. 2011. Microbial residues as indicators of soil restoration in South African secondary pastures. Soil Biol Biochem 43:787–94.

Lee J, Hopmans JW, Rolston DE, Baer SG, Six J. 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. Agric Ecosyst Environ 134:251–6.

Lobe I, Amelung W, Du Preez CC. 2001. Losses of carbon and nitrogen with prolonged arable cropping from sandy soils of the South African Highveld. Eur J Soil Sci 52:93–101.

Lobe I, Bol R, Ludwig B, Du Preez CC, Amelung W. 2005. Savanna-derived organic matter remaining in arable soils of the South African Highveld long-term mixed cropping: Evidence from C-13 and N-15 natural abundance. Soil Biol Biochem 37:1898–909.

Lobe I, Du Preez CC, Amelung W. 2002. Influence of prolonged arable cropping on lignin compounds in sandy soils of the South African Highveld. Eur J Soil Sci 53(4):553–62.

Low AJ. 1972. The effect of cultivation on the structure and other physical characteristics of grassland and arable soils. J Soil Sci 23:363–80.

Low AB, Rebelo AG. 1996. Vegetation of South Africa, Lesotho, and Swaziland. Pretoria: Department of Environmental Affairs & Tourism.

Mahaming AR, Mills AAS, Adl SM. 2009. Soil community changes during secondary succession to naturalized grasslands. Appl Soil Ecol 41:137–47.

Mann LK. 1986. Changes in soil carbon storage after cultivation. Soil Sci 142:279–88.

Matamala R, Jastrow JD, Miller RM, Garten CT. 2008. Temporal changes in C and N stocks of restored prairie: implications for C sequestration strategies. Ecol Appl 18:1470–88.

McCulley RL, Burke IC, Nelson JA, Lauenroth WK, Knapp AK, Kelly EF. 2005. Regional patterns in carbon cycling across the Great Plains of North America. Ecosystems 8:106–21.

McKinley VL, Peacock AD, White DC. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biol Biochem 37:1946–58.

McLauchlan KK, Hobbie SE, Post WM. 2006. Conversion from agriculture to grassland builds soil organic matter on decadal timescales. Ecol Appl 16:143–53.

O'Brien SL, Jastrow JD. 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. Soil Biol Biochem 61:1–13.

Oades JM, Waters AG. 1991. Aggregate hierarchy in soils. Aust J Soil Res 29:815–28.

Olsson PA. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. Fems Microbiol Ecol 29:303–10.

Parton WJ, Stewart JWB, Cole CV. 1988. Dynamics of C, N, P and S in grassland soils—a model. Biogeochemistry 5:109–31.

Paustian K, Andren O, Janzen HH, Lal R, Smith P, Tian G, Tiessen H, Van Noordwijk M, Woomer PL. 1997. Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use Manag 13:230–44.

Preger AC, Kosters R, Du Preez CC, Brodowski S, Amelung W. 2010. Carbon sequestration in secondary pasture soils: a chronosequence study in the South African Highveld. Eur J Soil Sci 61:551–62.

SAS/STAT(R). 2014. 9.22 User's Guide. http://support.sas.com/documentation/cdl/en/statug/63347/HTML/default/viewer.htm#statug_nlin_sect037.htm. Accessed on Oct 19 2014.

Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. 2001. Microbial population structures in soil particle

size fractions of a long-term fertilizer field experiment. Appl Environ Microbiol 67:4215–24.

Silver WL, Miya RK. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407–19.

Six J, Conant RT, Paul EA, Paustian K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155–76.

Six J, Frey SD, Thiet RK, Batten KM. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70:555–69.

Six J, Paustian K, Elliott ET, Combrink C. 2000. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci Soc Am J 64:681–9.

Sørenson LH. 1972. Stabilization of newly formed amino-acid metabolites in soil by clay minerals. Soil Sci Soc Am J 114:5–11.

Stewart CE, Paustian K, Conant RT, Plante AF, Six J. 2007. Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86:19–31.

SYSTAT Software Incorporated. 2002. TableCurve 2D Version 5.01 for Windows. Richmond: Systat Software Inc.

SYSTAT Software Incorporated. 2008. SigmaPlot Version 11.0. San Jose: Systat Software Incorporated.

Tisdall JM, Oades JM. 1982. Organic matter and water-stable aggregates in soils. J Soil Sci 33:141–63.

Vågen TG, Lal R, Singh BR. 2005. Soil carbon sequestration in sub-Saharan Africa: A review. Land Degrad Dev 16:53–71.

van der Wal A, van Veen JA, Smant W, Boschker HTS, Bloem J, Kardol P, van der Putten WH, de Boer W. 2006. Fungal biomass development in a chronosequence of land abandonment. Soil Biol Biochem 38:51–60.

West TO, Six J. 2007. Considering the influence of sequestration duration and carbon saturation on estimates of soil carbon capacity. Clim Change 80:25–41.

Zelles L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35:274–94.