

# Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration



Steven T. Rosenzweig<sup>a,b,\*</sup>, Michael A. Carson<sup>a</sup>, Sara G. Baer<sup>c</sup>, John M. Blair<sup>a</sup>

<sup>a</sup> Division of Biology, Kansas State University, Manhattan, KS 66506, USA

<sup>b</sup> Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523, USA

<sup>c</sup> Department of Plant Biology and Center for Ecology, Southern Illinois University, Carbondale, IL 62901, USA

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## ABSTRACT

Understanding the dynamics of soil C and N pools and fluxes following grassland restoration in formerly cultivated soils is needed to assess the capacity of this land-use change to return soil properties and function to pre-disturbance conditions. We examined changes in physical (bulk density) and chemical (pH, available P and N, and total stocks of C and N) properties, microbial biomass C and N, and transformation rates of C and N in soil (0–10 cm depth) across a 35-year chronosequence of prairie restorations and never-cultivated (native) prairie to evaluate the rate and extent to which sowing native perennial plants into formerly cultivated soils returns soil to steady state conditions. Bulk density, available P, and extractable inorganic N decreased exponentially across the chronosequence. Soil pH increased linearly across the chronosequence to exceed that in native prairie soil. Total C and N stocks in cultivated soil were <50% that of native prairie, and increased at rates of 26.2 g C m<sup>-2</sup> year<sup>-1</sup> and 1.68 g N m<sup>-2</sup> year<sup>-1</sup> across the chronosequence. In the oldest restoration, total C and N stocks were 55% and 41% higher than the cultivated soil, respectively. Recovery of C and N stocks to levels comparable to native prairie soil was estimated to take 350 years. Microbial biomass C and N increased 5-fold. Potential C mineralization rate was comparable to native prairie soil following two decades of restoration, and exceeded native prairie in the 35-years restoration. In situ soil CO<sub>2</sub> efflux reached equilibrium in <30 years. Potential net N mineralization rate, however, did not exhibit a directional change across the restoration chronosequence, but was lowest in the oldest restored grasslands. Thus, cessation of tillage and sowing native vegetation leads to rapid decreases in available N and P, and promotes rapid (decadal scale) recovery of labile soil organic matter pools, but recovery of total C and N pools requires several centuries. This study suggests that soil properties related to nutrient retention and regulation of nutrient availability can be reestablished within the first few decades of restoration, while C sequestration will continue on a longer time scale.

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## 1. Introduction

Ecosystem functions and services provided or supported by soils (e.g., primary productivity, water infiltration, nutrient abatement, erosion control, greenhouse gas mitigation, and habitat for biodiversity) are typically compromised during land conversion to agriculture (Wall, 2012). In North America, high soil fertility and rainfall sufficient to support plant production incentivized widespread conversion of the tallgrass prairie ecosystem to

agriculture (Samson and Knopf, 1994), a phenomenon that also has occurred in many other grasslands globally (Ellis and Ramankutty, 2008). Land conversion to agriculture has left only ca. 4% of North America's original 66 million hectares of tallgrass prairie unplowed (DeLuca and Zabinski, 2011; Samson and Knopf, 1994). Cultivation of grassland depletes soil organic matter (Haas et al., 1957; Knops and Tilman, 2000; Mann, 1986; Schlesinger, 1986; Steenwerth et al., 2002), alters the microbial community (Allison et al., 2005; Jangid et al., 2010), accelerates nutrient cycling (Burke et al., 1995), reduces nutrient retention (Kemp and Dodds, 2001), and compacts soil (Murphy et al., 2004). Restoring agricultural land to grassland is a common practice to improve soil properties and functions degraded through cultivation. Knowledge of the extent to which grassland restoration promotes soil recovery to pre-disturbance conditions and the timescale over

\* Corresponding author at: Colorado State University, 1107 Campus Delivery, Fort Collins, CO 80523, USA.

E-mail addresses: [steven.t.rosenzweig@gmail.com](mailto:steven.t.rosenzweig@gmail.com) (S.T. Rosenzweig), [sgbaer@siu.edu](mailto:sgbaer@siu.edu) (S.G. Baer), [jblair@ksu.edu](mailto:jblair@ksu.edu) (J.M. Blair).

which this occurs is needed for many soil, vegetation, and climate conditions to predict the potential for conservation practices to reverse soil degradation and reinstate ecosystem services (Baer et al., 2012; Dodds et al., 2008).

Agricultural conversion of grasslands results in large changes in C cycling and storage. In tallgrass prairie, dominant perennial grasses contribute large amounts of organic matter to soil through extensive root systems and high above- and below-ground productivity (Risser et al., 1981). This SOM pool supports a large and diverse soil microbial and faunal community, and contributes to the tight cycling of key nutrients such as nitrogen (N) and phosphorus (P) (Dell and Rice, 2005; McCulley et al., 2009). Tillage results in rapid SOM loss, and grassland conversion to cropland in the United States alone is estimated to have transferred 993 Tg of C to the atmosphere as CO<sub>2</sub> (Conant et al., 2001; Kern, 1994). Restoring former agricultural fields to perennial grasslands results in accrual of new plant-derived C into SOM pools. The active C pool generally increases in response to grassland restoration (Allison et al., 2005; Baer et al., 2000, 2002; Staben et al., 1997). Total soil C and N pools, however, change more slowly (Baer et al., 2002, 2010; Brye et al., 2002; Hansen and Gibson, 2014; Jangid et al., 2010; Matamala et al., 2008; McLaughlan et al., 2006), but accrual rates are highly variable (McLaughlan, 2006). Studies that have modeled time to reach steady state estimate less than 150 (Baer et al., 2010) to more than 400 years (Matamala et al., 2008) of restoration will be required to reach soil C stocks comparable to native prairie. A better understanding of the dynamics of soil C and N pools and fluxes in response to restoration is needed to assess the rates and extents to which soil function can be improved by restored grasslands.

Tillage and fertilizer application promote nutrient losses from agricultural lands, with far-reaching implications for environmental and human health (Gardner and Drinkwater, 2009). Tallgrass prairie is a N-limited ecosystem where nutrients mineralized in the soil are quickly immobilized by the microbial biomass or taken up by plants (Dell et al., 2005). Conventional agricultural practices open N cycling through inputs of N and these soils generally contain higher levels of inorganic N, susceptible to loss through overland flow and leaching (Gruber et al., 2011; Kemp and Dodds, 2001). Increasing root inputs, microbial biomass, and plant demand for N during restoration promotes progressive N limitation in restored soil (Baer and Blair, 2008) and several studies have demonstrated rapid reduction and lower N availability in restored relative to cultivated soil (Baer et al., 2000; Karlen et al., 1999; Staben et al., 1997).

In addition to altering nutrient cycling, cultivation degrades soil structure through the physical disruption of aggregates, and grassland restoration can reverse this condition. Development of root systems and microbial populations, particularly fungi, promote macroaggregate development in soils containing adequate clay content (Bach et al., 2010; O'Brien and Jastrow, 2013). The incorporation of smaller aggregates into macroaggregates reduces soil bulk density (Bach et al., 2010; Matamala et al., 2008). Bulk density is a key soil property that indicates water- and air-filled pore space, biological activity, root penetration, and physical function (Karlen et al., 1997), and been shown to decrease with grassland restoration (Baer et al., 2002; Matamala et al., 2008).

We used a chronosequence approach to quantify change in soil physical, chemical, and biological properties and processes during restoration and model recovery dynamics to predict time for these soil attributes to return to levels representative of uncultivated prairie, or a new equilibrium. This approach has been useful for quantifying soil change over decadal time scales and predicting recovery to undisturbed conditions (Bach et al., 2010; Baer et al., 2002, 2010; Jastrow, 1987; Matamala et al., 2008; McLaughlan et al., 2006). We hypothesized that bulk density would be highest

in cultivated (unrestored) soil and decrease following cessation of tillage and restoration of native vegetation. We also hypothesized that total soil C and N stocks would increase on a decadal timescale as a function of time since restoration, but modeled recovery to steady state would require centuries. We predicted microbial C and N, with faster turnover rates, and potential C mineralization rates would become representative of never-cultivated prairie within two decades in response to increasing root biomass and organic matter inputs, and in situ soil respiration would reflect labile C dynamics. Lastly, we hypothesized that plant-available nutrients and potential net N mineralization rates would decline over time as a result of increased plant uptake and microbial immobilization of nutrients (Baer et al., 2002; Karlen et al., 1999).

## 2. Methods

### 2.1. Study area

Study sites consisted of a conventionally cultivated wheat field, never-cultivated (native) prairie, and 6 previously cultivated fields restored to tallgrass prairie vegetation at (or within 4 km of) the Konza Prairie Biological Station (KPBS) and Long-Term Ecological Research site in the Flint Hills of eastern Kansas, USA (39°05'N and 96°35'W). Prior to cultivation, vegetation at all study sites was dominated by perennial warm-season (C<sub>4</sub>) grasses representative of native lowland tallgrass prairie characteristic of this region (Knapp et al., 1998a). All sites were located within 1 km of one another, with the exception of the 26-year old site located approximately 4 km northeast of KPBS. The climate is temperate continental; mean annual precipitation is 834 mm, with ca. 75% occurring in spring and early summer, and mean monthly air temperature varies from −3 °C in January to 27 °C in July (Hayden, 1998).

The chronosequence consisted of agricultural fields sown to native grasses and forbs 1, 3, 7, 15, 26, and 35-year ago and a cultivated field to represent initial conditions. The never cultivated (“native”) prairie was included to estimate steady state conditions. The same number of sites was used to model soil change across a prairie restoration chronosequence in Illinois (Jastrow, 1987). The active and formerly cultivated fields used in this study have been in agricultural production since the early 20th Century. Since 1976, the fields have been used for *Triticum aestivum* (winter wheat), *Zea mays* (maize), *Sorghum bicolor* (grain sorghum), or *Glycine max* (L.) (soybean) production and fertilized with liquid ammonium phosphate (S. Runquist and T. Van Slyke, personal communication). The cultivation history of the 35-year old site is unknown, but was likely similar. Soil at the sites are primarily fine silt, mixed, superactive, and mesic Pachic Argiudolls formed by colluvial and alluvial deposits. We selected fields with similar texture (Reading clay loams and Chase silty clay loams), slope (<2%), and management.

Grasslands were restored with dominant prairie grasses and prairie forbs at all sites. Plots restored for 1, 3, 7, 15 year were sown with 600 live seeds m<sup>-2</sup> in grass:forb ratios ranging from 60:40 to 70:30. Seeds of all species were hand broadcast into fields restored for 7 year or less. Forbs were hand broadcast in the 15 year restored field, but grasses were planted with a grass drill. Less is known about the composition of species introduced into the 26 and 35 year old restorations, but locally-collected prairie hay was applied as a seed source. All sites restored for >3 year and native prairie were managed by burning in 1–2 year intervals. Prescribed spring burning is a common management practice to maintain grassland in this region. In the year of study, all sites except the 0, 1, and 7 year restored sites were burned prior to the growing season, two months before sampling. Previous research in these grasslands indicates that the cumulative long-term effects of frequent burning

have larger effects on plant community structure and soil processes than short-term responses to individual fires (Blair, 1997; Knapp et al., 1998b), suggesting that differences in burning in the year of measurement contributed little to results in this study.

## 2.2. Sampling

Four 4 m × 4 m plots were delineated in each field. In June of 2013, we removed ten soil cores (10 cm deep × 2 cm diameter) randomly from each plot and composited them by plot for subsequent processing. Bulk density was sampled to a depth of 10 cm using a 5.5 cm diameter hammer corer. Two bulk density cores were removed from each plot. In the laboratory, each bulk density core was weighed and passed through an 8 mm sieve during the course of removing roots. Two 30 g subsamples were weighed, dried at 105 °C, and reweighed to determine the mass (g) of dry soil per unit volume (cm<sup>3</sup>). We used the minimum equivalent mass (Lee et al., 2009), as used by Baer et al. (2010), to convert concentrations of C and N to stocks, pools, and transformation rates expressed on an aerial basis (g m<sup>-2</sup>).

## 2.3. Soil properties

Composited field-moist soil samples from individual plots were crumbled by hand, passed through a 4 mm sieve, root-picked for 12 min, and stored at 4 °C. A 125 g subsample of soil from each plot was air-dried and analyzed for pH (1:1 slurry of 10 g of soil and deionized water), available P (Mehlich-3 procedure), cation exchange capacity (displacement method with saturating ammonium acetate), and soil texture (hydrometer method following dispersal with sodium hexametaphosphate) using standard methods at the Kansas State University Soil Testing Lab. Soil water content of samples was calculated by drying a 55 g field-moist subsample from each plot at 60 °C for 2 days. Dried soil was finely ground in a SPEX 8400 Mixermill and used to determine percent C and N by dry combustion coupled with gas chromatography on a Flash EA 1112C/N analyzer (Thermo Fisher Scientific, Waltham, MA).

Soils were adjusted to 50% water holding capacity (WHC) prior to measuring microbial biomass and C and N mineralization potentials. To determine WHC, approximately 20 g of field-moist soil from each plot was saturated in funnels with Whatman No. 1 filter paper, and water content was determined after 16 h of gravitational drainage in sealed coolers to maintain humidity.

## 2.4. Microbial biomass carbon and nitrogen

Microbial biomass was determined using the fumigation direct-extraction procedure as the difference between fumigated and non-fumigated samples (Brookes et al., 1985; Vance et al., 1987). Duplicate non-fumigated samples of field-moist soil (ca. 12 g) from each plot were extracted with 75 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub> on an orbital shaker (New Brunswick Scientific, Edison, New Jersey) at 200 rpm for 1 h. Extracts were allowed to settle for 45 min prior to filtration through a 0.4 mm polycarbonate filter, and stored at 4 °C for 11 days. A second set of soil samples was fumigated by boiling ethanol-free chloroform under vacuum in a desiccator for 2 min, and then repeating. Fumigated samples remained under vacuum in the dark for 48 h then alternately vented and placed under vacuum four times (5 min each) to remove chloroform prior to extraction with K<sub>2</sub>SO<sub>4</sub>. Organic C in the extracts was measured with a Shimadzu TOC-L fitted with a high salt kit. Prior to analysis, K<sub>2</sub>SO<sub>4</sub> extracts were hand diluted 4:1 with DI water to both reduce salt concentration and ensure sufficient extract for microbial biomass nitrogen (MBN) analysis.

Microbial biomass N was determined using a persulfate digestion of the same K<sub>2</sub>SO<sub>4</sub> extracts used for MBC measurements. Digestion samples (16 ml) were made in 25 ml digestion tubes with a 1:1:2 ratio of 0.5 M K<sub>2</sub>SO<sub>4</sub> extract to DI water to persulfate cocktail made with low N chemicals: 100 g potassium persulfate (Sigma-Aldrich 60489), 60 g boric acid, 30 g NaOH (Fisher S3185-500) in 2 L of DI water. Samples were autoclaved with standards, spikes, and wash/baseline solution (all with molar equivalents of solutes) for 40 min at 120 °C, cooled, and refrigerated for storage. Samples were analyzed for nitrate on an OI Analytical Flow Solution IV+ (College Station, TX).

## 2.5. Potential C mineralization and net N mineralization rates

Potential net N mineralization rate was measured using a short-term (30 days) laboratory incubation (Robertson et al., 1999) using soil adjusted to 50% WHC. Both time zero and incubated subsamples (12 g field-moist) were weighed into 125 ml Erlenmeyer flasks. Flasks were covered with Parafilm and pre-incubated for seven days in a dark room at constant temperature. At the onset of the incubation, inorganic N was extracted to quantify initial N availability. Subsamples were extracted with 50 ml of 2 M KCl on an orbital shaker (New Brunswick Scientific, Edison, New Jersey) at 200 rpm for 1 h. Extracts were allowed to settle for 45 min prior to filtration through a 0.4 mm polycarbonate filter. Flasks containing subsamples for the incubation were sealed in quart mason jars containing 5 ml water to maintain humidity. After the 30 days incubation, the final inorganic N was extracted from soil. Inorganic N concentrations were determined colorimetrically on an OI Analytical Flow Solution IV+ autoanalyzer (College Station, TX). Net nitrification, ammonification, and N mineralization rates were calculated from the final minus initial concentrations of NO<sub>3</sub>-N, NH<sub>4</sub>-N, and NO<sub>3</sub> + NH<sub>4</sub>-N, respectively, divided by 30.

Potential C mineralization rates were measured from the same subsamples used to quantify net N mineralization. Headspace gas was extracted through rubber septa in the lid of each mason jar. On each sampling date (weekly throughout the 30 days incubation period), headspace gas was first homogenized using a 10 ml syringe, and a 0.5 ml sample was withdrawn with a 1 ml syringe and analyzed for CO<sub>2</sub>-C concentration on a Shimadzu GC-8A (Kyoto, Japan). Following each measurement, lids were removed and headspace was allowed to equilibrate with the atmosphere for 30 min before being resealed.

## 2.6. In situ soil CO<sub>2</sub> efflux

Field soil CO<sub>2</sub> efflux was measured every two weeks using a LiCor-8100 soil respiration system (Lincoln, NE, USA). Two PVC collars (10 cm diameter × 9 cm deep) were inserted approximately 7 cm into the soil at random locations in corners of every plot, and allowed to settle for one week prior to starting measurements. Two LiCor-8100 portable infrared gas analyzers fitted with temperature probes were used to measure soil CO<sub>2</sub> efflux and soil temperature (10 cm). All measurements were done between 1100 and 1500 h.

## 2.7. Statistical analyses

We used the average response from the four subplots for modeling soil recovery across the chronosequence. Standard errors are presented to show within-field variability. We used Table Curve 2D 5.01 (SYSTAT Software Incorporated, 2002) to evaluate simple equations (2–3 parameter) that best described the relationship between each soil response variable and restoration age using model rankings generated by coefficients of determination and significance level of the model fit. Total C and N stocks in the restored plots were best described by exponential growth models,

**Table 1**

Soil texture and cation exchange capacity (CEC) in a cultivated agricultural site (Years Restored = 0), a chronosequence of restored grasslands, and native prairie (NP). All values are means of four plots for each site.

Years restored	(%) Sand	(%) Silt	(%) Clay	CEC (cmol/kg)
0	16.0	52.5	31.5	24.9
1	16.5	51.0	32.5	22.3
3	18.5	48.5	33.0	23.0
7	17.0	55.5	27.5	18.4
15	18.0	58.5	23.5	16.5
26	23.0	46.0	31.0	21.9
35	18.5	52.0	29.5	23.4
NP	22.0	58.0	20.0	20.9

but this type of increase in total C and N would not be expected over the long-term because C saturation theory predicts C and N accrual rates will decrease over time (Stewart et al., 2007). For this reason, we included the never-cultivated prairie assigned to an age of 1000 year to model recovery of total C and N stocks, which were then best described by exponential rise to maximum models (Baer et al., 2010; 2015; Matamala et al., 2008; O'Brien and Jastrow, 2013). Native prairie was not included in models to evaluate recovery of bulk density, P availability, inorganic N availability, pH, potential C mineralization rate, MBC, MBN, and soil CO<sub>2</sub> efflux because these variables were expected to show more recovery on a decadal time scale than total C and N stocks. Time to achieve equilibrium (defined as 99% of the model limit) was estimated using the following equation,

$$t = \log[0.01S_e / (S_e - S_o)] * (-1/k),$$

where  $S_o$  is the parameter estimate in the currently cultivated plot,  $S_e$  is the steady state condition defined as 99% of the model limit,  $k$  is the rate constant defined in the model, and  $t$  is time. All figures were created in R (R Core Team, 2013).

### 3. Results

Soil texture was similar among all sites and CEC ranged from 16.5 to 24.9 cmol/kg (Table 1), with no apparent directional change in texture or CEC across the chronosequence. Bulk density was highest in the agricultural soil and decreased exponentially with restoration age, with an estimated time to reach equilibrium of 17 years

(Fig. 1A). Soil pH increased linearly over the chronosequence and surpassed that of the never-cultivated prairie, which was intermediate of the oldest and youngest restored prairies (Fig. 1B). Available nutrient pools were significantly affected by restoration. Both extractable N and P declined exponentially with grassland age, reflecting increasing nutrient limitation in older restored prairies. Extractable inorganic N and plant-available P were highest in the agricultural soil, and decreased to equilibrium at 7 and 35 years, respectively (Fig. 2).

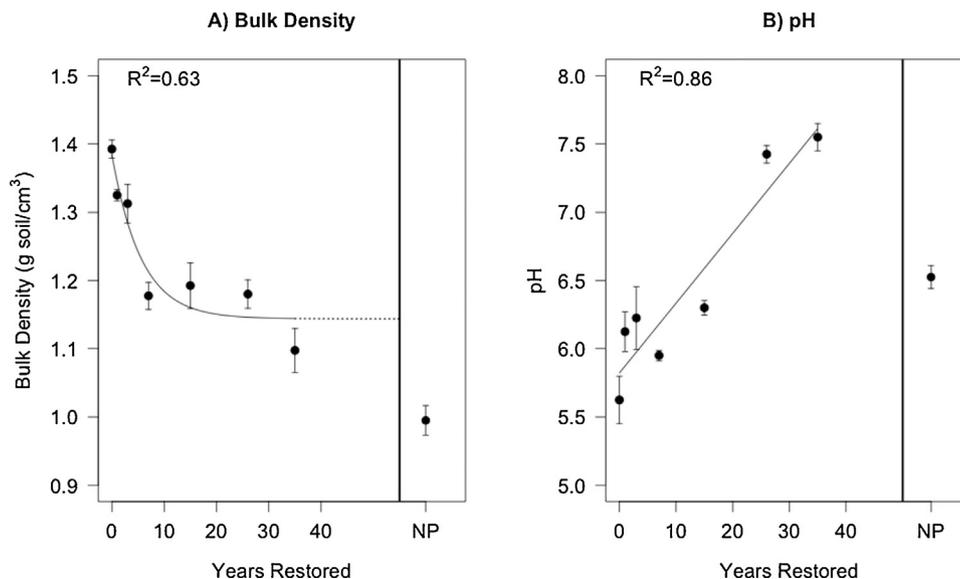
Total C and N stocks in the agricultural soil were 1670 g C m<sup>-2</sup> and 145 g N m<sup>-2</sup>, and were only 46% (C) and 49% (N) that of the native prairie soil, respectively. Total C and N increased at rates of 26.2 g C m<sup>-2</sup> year<sup>-1</sup> and 1.68 g N m<sup>-2</sup> year<sup>-1</sup> over the 35 year restoration chronosequence to 69% (C) and 71% (N), respectively, of that in prairie that had never been cultivated. Time estimated to reach levels in extant native prairie based on the exponential rise to maximum model for total soil C and N stocks was 333 and 351 years, respectively (Fig. 3A and B). There were no significant trends in soil C:N ratio with restoration, but C:N was highest in the oldest restoration and native prairie (Fig. 3C).

Microbial biomass and potential C mineralization rates increased in response to restoration. Microbial biomass C and N increased linearly across the chronosequence, achieving a 5-fold increase by the 35th year of restoration over levels in the cultivated field (Fig. 4A, 4B). Potential C mineralization rate in restored prairie soil exceeded that in native prairie soil (1.09 g CO<sub>2</sub>-C/m<sup>2</sup>/d) within three decades (Fig. 5A). There was no directional change in net N mineralization rate across the restoration chronosequence, but net N mineralization was lowest in the oldest restored grasslands (Fig. 5B).

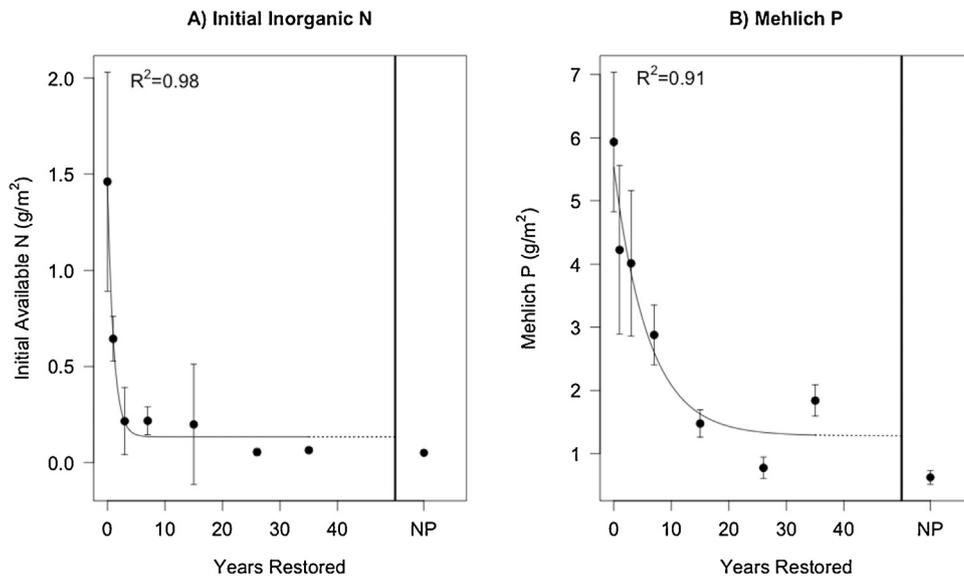
In situ soil CO<sub>2</sub> efflux varied across sample dates during the growing season, depending on plant phenology and environmental conditions, but was always lowest in the agricultural soil and generally greatest in the native prairie and oldest restoration sites (seasonal data not shown). When averaged across sample dates, soil CO<sub>2</sub> efflux increased with time since restoration, and the time to reach equilibrium was estimated to be 28 years (Fig. 5C).

### 4. Discussion

Several physical properties of soil changed in response to restoration within a decadal time frame. This is one of first studies



**Fig. 1.** Modeled changes in soil (A) bulk density and (B) pH across the restoration chronosequence and native prairie (NP). Data points are means  $\pm$  SE of 4 replicate plots within each site. Lines fit to data represent significant models ( $p < 0.05$ ): soil bulk density =  $1.14 + 0.24e^{-0.179x}$ ; pH =  $5.82 + 0.0512x$ .

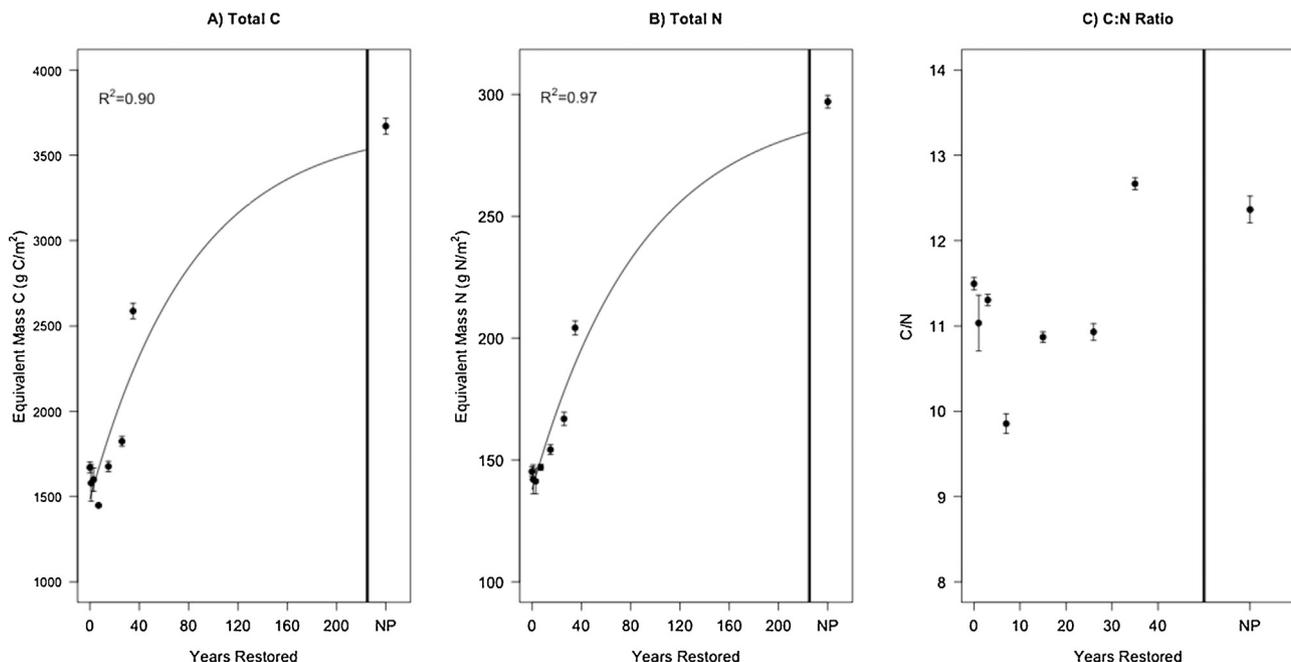


**Fig. 2.** Changes in (A) extractable inorganic N and (B) Mehlich P across the restoration chronosequence and native prairie (NP). Data points are means  $\pm$  SE of 4 replicate plots within each site. Some error bars are too small to see. Lines fit to data represent significant models ( $p < 0.05$ ): extractable inorganic N =  $0.135 + 1.32e^{-0.948x}$ ; Mehlich P =  $1.28 + 4.26e^{-0.145x}$ .

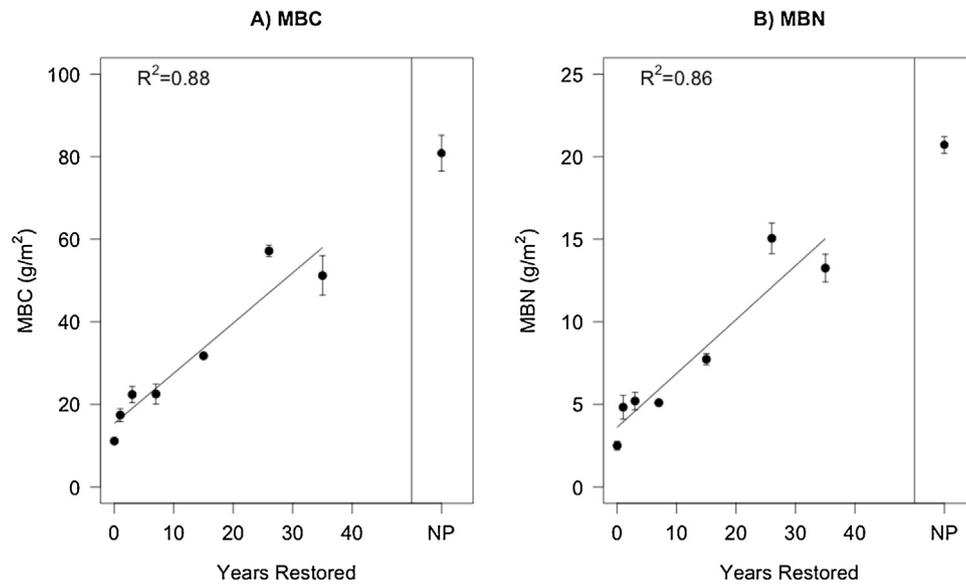
to demonstrate relatively rapid reduction in soil bulk density across a grassland restoration chronosequence. In addition to promoting mineralization and loss of stored organic matter, tillage degrades soil structure, resulting in more compact soil with a higher bulk density. The observed decrease in bulk density with grassland restoration age reflects improvement of soil structure over time (Bach et al., 2010; Murphy et al., 2004). Lower bulk density is generally correlated with greater water infiltration rates, and reduced surface runoff and erosion (Foster et al., 1985).

Restoration also altered soil pH. Long-term N fertilizer applications promote nitrification and  $H^+$  production (Karlen et al., 1999). Soil acidity decreased as a function of time since restoration to more neutral levels after two decades of grassland

restoration. The pH of the uncultivated prairie soil, however, was intermediate of the range found in the restored sites and consistent with other estimates of pH at Konza Prairie (A. Cann, unpublished data). To our knowledge, this is the first study to demonstrate an increase in pH in response to prairie restoration, which is important because soil pH is one of the strongest controls of microbial community diversity and richness (Fierer and Jackson, 2006). The increase in pH from acidic to neutral levels over the restoration period may be an important driver of the change in microbial community structure that has been observed in other restoration chronosequences (Allison et al., 2005; Jangid et al., 2010), though additional study is required to assess the relative role of pH in this context.



**Fig. 3.** Changes in (A) total soil C stock, (B) total soil N stock, and (C) C:N ratio across the restoration chronosequence and native prairie (NP). Data points are means  $\pm$  SE of 4 replicate plots within each site. ( $p < 0.05$ ). Lines fit to data represent significant models ( $p < 0.05$ ): total soil C stock =  $1480 + 2200(1 - e^{-0.0120x})$ ; total soil N stock =  $138 + 160(1 - e^{-0.0112x})$ .

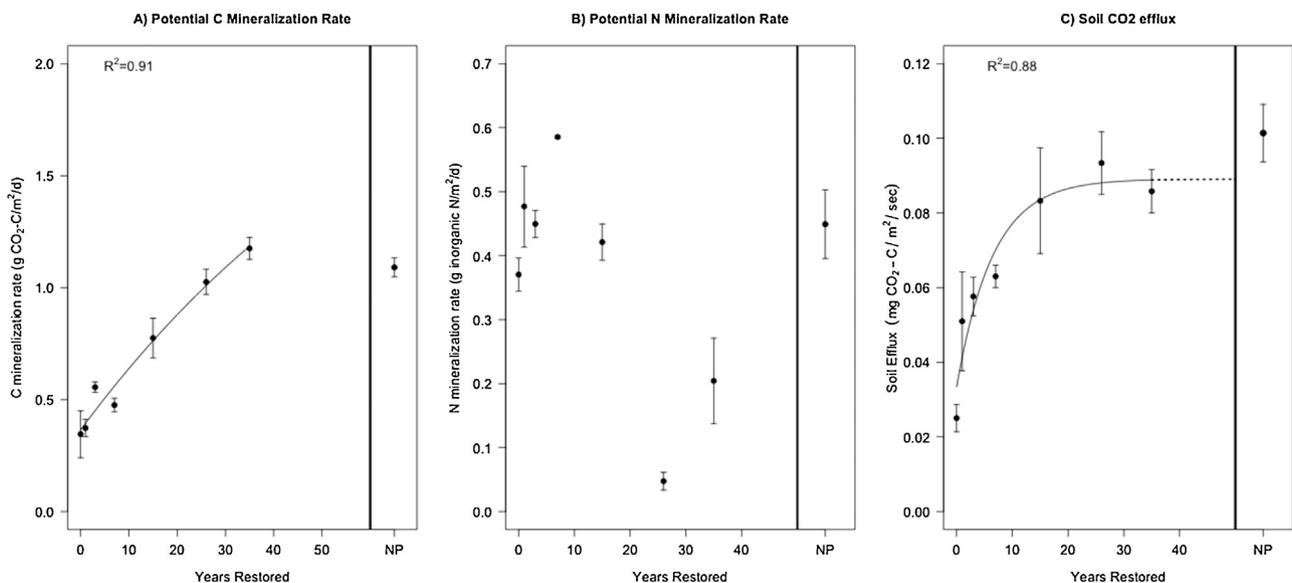


**Fig. 4.** Modeled temporal changes in (A) microbial biomass carbon and (B) microbial biomass nitrogen over the restoration chronosequence and native prairie (NP). Symbols are means  $\pm$  SE of 4 replicate plots within each site. Lines fit to data represent significant models ( $p < 0.05$ ):  $MBC = 15.4 + 1.21x$ ;  $MBN = 3.61 + 0.327x$ .

Cation exchange capacity (CEC) influences soil nutrient retention, and has been shown to increase with active soil organic matter content in restored grasslands (Karlen et al., 1999). Despite increases in all C pools with time since restoration, we observed no change in CEC across the chronosequence. We attribute this to the high clay content of soil in all sites used in the chronosequence. Further, CEC was not reduced by agricultural practices, as might be expected with loss of soil organic matter. In fact, the cultivated soil contained the highest CEC. This finding is consistent with another grassland chronosequence study in soil with relatively high clay content (Baer et al., 2002).

Loss of soil organic C in response to cultivating grassland ranges from 23 to 70 percent in the surface 20 cm of soil in the Great Plains of North America (Baer et al., 2002; Mann, 1986; Parton et al., 1988), resulting in a capacity for soil to accrue C and N. Total C and N stocks in the active agricultural field and recently restored

grasslands in this study were less than 50% of those in native prairie soil. Total C and N stocks increased across the chronosequence, but were variable in early-restored sites. We attributed this variability to differences in plant species composition among the early restorations, as the early successional annual species *Coryza canadensis* dominated the year 1 restoration, and native grasses were more prevalent in the year 3 restoration (Scott, 2015). Perennial grasses produce greater root biomass than annual species, thus contributing more C and N to the soil (Risser et al., 1981). Although restoration can bypass or shorten the early stages of succession,  $C_4$  grasses can take  $\sim 6$  year to become well established as the dominant vegetation (Baer et al., 2002). Despite this early variability, total C and N stocks increased linearly across the chronosequence and were within the range of previously estimated C and N accrual rates ( $\sim 20\text{--}60 \text{ g C m}^{-2} \text{ year}^{-1}$  and  $\sim 1\text{--}3 \text{ g N m}^{-2} \text{ year}^{-1}$ ) from the onset of restoration



**Fig. 5.** Modeled temporal changes in (A) potential C mineralization rate, (B) potential N mineralization rate, and (C) in situ soil CO<sub>2</sub> efflux across the restoration chronosequence and native prairie (NP). Data points are means  $\pm$  SE of 4 replicate plots within each site. Lines fit to data represent significant models ( $p < 0.05$ ): potential C mineralization rate =  $0.36 + 2.23(1 - e^{-0.0131x})$ ; soil CO<sub>2</sub> efflux =  $0.033 + 0.056(1 - e^{-0.154x})$ .

(Baer et al., 2002; Matamala et al., 2008; McLauchlan et al., 2006; Post and Kwon, 2000), but lower and higher rates have also been reported (McLauchlan, 2006). Variability in soil C and N accrual rates among studies may result from differences in initial C saturation deficit, restoration methods, environmental conditions during the restoration, and management of restored grasslands (Jones, 2010).

Carbon saturation theory predicts C and N accrual rate will decrease over time (Knops and Tilman, 2000; Matamala et al., 2008; Stewart et al., 2007). Our estimate of ca. 350 years for total C and N stock to recover to steady state is consistent with other long-term estimates of total C and N recovery (Knops and Tilman, 2000; Matamala et al., 2008). This suggests a slowly increasing SOM pool acts as a C sink until a new equilibrium between C inputs and C losses is reached. Long-term protection of soil organic matter will, however, depend on the degree of protection from decomposition through mineral sorption to clays (Sørensen, 1972), recalcitrance (Krull et al., 2003), and formation of stable soil aggregates (Elliott, 1986; Six, 2000; Tisdall and Oades, 1982). Additionally, the observed increase in total N in the upper 10 cm of soil through time probably reflects an increase in soil organic C, which results in increased N limitation leading to fewer N losses from the system. This assumption is supported by the increases in total C and MBC, and decreases in extractable inorganic N across the chronosequence.

The reduction in N availability, growing microbial biomass, and increasing soil C:N ratios (>12 in the oldest restored and native prairie sites) suggest progressive N limitation develops during grassland restoration on formerly cultivated soil. This is consistent with N limitation and high potential for N immobilization in never cultivated prairie soil (Blair et al., 1998; Dell et al., 2005). Developing N limitation in these restored grasslands is likely due to increasing quantity of low quality organic matter inputs from roots as restored prairies become dominated by C4 grasses (Baer et al., 2002, 2010).

While tallgrass prairie soil contains large stores of organic N, plant-available inorganic forms occur in low concentrations due to relatively low rates of decomposition and mineralization, and a high potential for microbial immobilization (Dell et al., 2005), resulting in N limitation (Blair et al., 1998). The high concentration of available inorganic N and P in the agricultural field was due to fertilization during agricultural management. The availability of N and P declined rapidly as a function of time since restoration, likely due to immobilization and assimilation by reestablishing microbial and plant communities, supporting a previous suggestion that progressive N limitation occurs as C accumulates during grassland restoration (Baer and Blair, 2008). High plant-available P could also contribute to the increasing N limitation in young restorations (Matamala et al., 2008). Lower potential N mineralization rates in the 26- and 35-year restorations may also be due to greater potential microbial N immobilization in soils from older restorations, as reflected by corresponding increases in MBN. The high potential for N retention within native prairies is also evident by the low levels of nutrient export from native prairie via stream flow (Dodds et al., 1996).

Native prairie soil supported the largest microbial biomass C and N pools, and we expected that this would result in low potential net N mineralization rates as a result of microbial demand for mineralized N (i.e., a high immobilization potential). Counter to our expectation, there were higher potential N mineralization rates in the native prairie relative to the older restored grasslands. This was consistent with other comparisons of restored grasslands to tallgrass prairie (Baer et al., 2002, 2010), and may reflect differences in root quality associated with significant changes in plant composition over time in restored prairie. Root biomass in never-cultivated prairie may have lower mean C:N

ratios than restored prairie (Baer et al., 2010) due to the greater presence of C<sub>3</sub> grasses and forbs in native prairie, which have roots with lower C:N than C<sub>4</sub> grasses (Craine et al., 2002). In addition, laboratory assays of N mineralization potentials reflect the net effects of several processes (ammonification, immobilization, nitrification), which can make them difficult to interpret.

The potential rate of C mineralization and in situ soil CO<sub>2</sub> flux increased steadily across the chronosequence. This indicates that the amount and turnover of labile organic C recovers quickly after restoration, presumably as a function of increased root biomass, labile C inputs, and associated microbial activity (Baer and Blair, 2008; Maher et al., 2010). The increase in in situ soil CO<sub>2</sub> flux is likely attributed to changes in soil aggregation coinciding with restoration. A complementary study of aggregate structure across this chronosequence shows the majority of soil C was associated with recovery of macroaggregates (Scott, 2015), which may be susceptible to greater microbial decomposition (Six et al., 2000). Although soil CO<sub>2</sub> efflux is a dynamic process, there was a strong relationship between soil CO<sub>2</sub> efflux and grassland age. This is similar to results reported by Guzman and Al-Kaisi (2010) and Maher et al. (2010), although our study extends the temporal scale of in situ respiration responses compared to earlier studies. The large increase in soil CO<sub>2</sub> efflux with time since restoration likely reflects the increasing size of the mineralizable pool of C as restoration proceeds (Baer and Blair, 2008), as well as increased root biomass (Maher et al., 2010). However, there is also likely to be increasing physical and chemical protection in hierarchical soil aggregates with time (O'Brien and Jastrow, 2013), which would contribute to increased C storage despite higher CO<sub>2</sub> efflux rates. Thus, our model prediction of greater potential C mineralization in restored compared to native prairie soil may reflect an overestimate of potential C mineralization rates. It is possible that the size of the labile C pool will decrease to a new steady state more representative of never cultivated prairie as SOM stabilization mechanisms develop.

In summary, this study contributes to a growing body of knowledge concerning the effects of re-establishing grassland on soil properties, microbial biomass and activity, and microbially mediated processes in formerly disturbed ecosystems. We show grassland restoration improves degraded soil structure, reduces soil acidity, increases microbial biomass, and increases soil respiration (in situ and laboratory soil CO<sub>2</sub> efflux) corresponding with exponential declines in available N and P on a fairly rapid (decadal) time scale. Time predicted for recovery of total C and N stocks, however, was much longer (century time scale). Changing belowground structure and function from agricultural to that more representative of prairie should sustain the productivity of prairie vegetation (Rice et al., 1998). Only very long-term studies will demonstrate whether extant prairie soil that has never been cultivated represents a valid target to assess soil recovery in response to restoration, or whether a legacy of cultivation reduces the capacity for soil and ecosystem function.

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