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Incorporation of Plant Residues into Soil Organic Matter Fractions With Grassland Management Practices in the North American Midwest

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Abstract

Disturbed grassland soils are often cited as having the potential to store large amounts of carbon (C). Fertilization of grasslands can promote soil C storage, but little is known about the generation of recalcitrant pools of soil organic matter (SOM) with management treatments, which is critical for long-term soil C storage. We used a combination of soil incubations, size fractionation and acid hydrolysis of SOM, [C], [N], and stable isotopic analyses, and biomass quality indices to examine how fertilization and haying can impact SOM dynamics in Kansan grassland soils. Fertilized soils possessed 113% of the C possessed by soils subjected to other treatments, an increase predominantly harbored in the largest size fraction (212–2,000 μm). This fraction is frequently associated with more labile material. Haying and fertilization/haying, treatments that more accurately mimic true management techniques, did not induce any increase in soil C. The difference in ^{15}N-enrichment between size fractions was consistent with a decoupling of SOM processing between pools with fertilization, congruent with gains of SOM in the largest size fraction promoted by fertilization not moving readily into smaller fractions that frequently harbor more recalcitrant material. Litterfall and root biomass C inputs increased 104% with fertilization over control plots, and this material possessed lower C:N ratios. Models of incubation mineralization kinetics indicate that fertilized soils have larger pools of labile organic C. Model estimates of turnover rates of the labile and recalcitrant C pools did not differ between treatments (65.5 ± 7.2 and 2.9 ± 0.3 μg C d^{-1}, respectively). Although fertilization may promote greater organic inputs into these soils, much of that material is transformed into relatively labile forms of soil C; these data highlight the challenges of managing grasslands for long-term soil C sequestration.

Key words: soil carbon; soil organic matter; grassland; land management; carbon sequestration; *Bromus inermis*; *Festuca arundinacea*; *Poa pratensis*.

Introduction

Recent analyses of the United States carbon (C) budget suggest that promoting soil C sequestration via land management strategies may be less effective at mitigating rising atmospheric CO_2 concentrations than increasing vehicular fuel efficiency (Jackson and Schlesinger 2004). Other investigators suggest that managing ecosystems for soil C sequestration offers valuable opportunities for partially offsetting fossil fuel C emissions, at least until C emissions are further curtailed (Follett

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2001; Lal 2004). Because of the significant quantities of soil C released as CO_2 when intact ecosystems are disturbed for agricultural use (Huggins and others 1998; Richter and others 1999; Post and Kwon 2000), soils in ecosystems recovering from such disturbances are often viewed as strong sinks for C relative to more steady state systems (Gebhart and others 1994; Huggins and others 1998; Kindscher and Tieszen 1998; Post and Kwon 2000; Williams and others 2004), such as native prairies.

Native prairies experienced dramatic C depletions when they were first plowed for agricultural use (Tiessen and Stewart 1983), as happened in the late nineteenth century and early twentieth century in North America. Approximately 3.7 Pg of C was released annually from worldwide agricultural expansion between 1860 and 1890, with the North American Great Plains contributing significantly to this flux (Wilson 1978). This quantity represents almost 60% the annual fossil fuel emissions in the 1990s of 6.3 Pg C y^{-1} (Schlesinger 1997). As a result of this massive efflux of C from the conversion of native systems to agriculture, soils in the Great Plains are thought to have high C sequestration potential (Huggins and others 1998; Conant and others 2001; Lal 2002).

Land managers in numerous Great Plains states are exploring how management practices on agricultural fields and grasslands could promote significant C storage in soils. For example, in addition to its obvious benefits for food and forage production, fertilization has been proposed as a technique for enhancing soil C storage (Rasmussen and Rohde 1988; Huggins and others 1998; Halvorson and others 1999; Conant and others 2001; Malhi and others 2003) because of its positive impact on above- and belowground production (Russell and Williams 1982). There is significant controversy over whether such policies would be effective. Studies of nitrogen (N) additions indicate that N fertilization can induce increases in C mineralization of recalcitrant C pools (Dijkstra and others 2005) or, alternatively, can promote soil organic matter (SOM) accumulations, often through changes in ligninase activity (Zech and others 1994, Dijkstra and others 2004). From an ecosystem perspective, C storage in soils is most useful if it results in long-term C sequestration, but little is known about how fertilization and other common land management techniques employed in grasslands impact long-term C storage. Such sequestration relies on the ability of ecosystems to process plant residues into SOM components possessing long turnover times.

To address this issue, we examined SOM properties from an experimental grassland in Northeast Kansas subjected to fertilization and haying treatments. We used a combination of long-term soil incubations and modeled parameter estimates from the resulting data, [C], [N], and $\delta^{13}C$ and $\delta^{15}N$ of bulk SOM and aggregate size fractions, and [C] and [N] of fractions from acid hydrolysis of soils to assess how these land management practices can impact the capacity of these soils to process and store organic matter. We also quantified C, N, cellulose, and lignin content of plant litterfall, and C and N content of root biomass in associated plots, as well as the $\delta^{13}C$ and $\delta^{15}N$ values of these materials to determine relationships between biomass quality indices and SOM storage properties. We use these techniques to explore the utility of grassland management practices as a means of sequestering C in long-term soil pools.

Materials and Methods

The study took place at an experimental grassland at the Kansas Field Station and Ecological Reserves (39°N, 94°W) (Fitch and Kettle 1988). The region has a temperate climate characterized by cold winters and hot summers, with a mean annual temperature of approximately 13°C. Mean annual precipitation totals 900 mm, and the growing season is approximately 185 days. The area represents the tallgrass prairie/forest ecotone, at the western edge of the eastern deciduous forest biome and the eastern edge of the tallgrass prairie region. Soils are Pawnee clay loams, classified as fine, montmorillonitic, mesic Aquic Argiudolls with high water holding capacity and natural fertility (USDA Soil Conservation Service 1977). Soil pH_{water} of soils at similar surrounding grassland sites ranges from 6.0 to 6.7, and no soil inorganic C is present, as determined by comparing C concentration and $\delta^{13}C$ data from acid-washed soils with non-acid-washed soils (S. A. Billings, unpublished data).

Most native prairie in the area was plowed for agricultural use by the early twentieth century, and many fields were subsequently used as hayfields, supporting non-native, cool-season grasses. The study site had been maintained as a cool-season grass hayfield since the mid-twentieth century, and may have been periodically fertilized until 1987. After that date, the study site was maintained solely by periodic mowing. Plant species include the non-native *Bromus inermis*, *Festuca arundinacea*, and *Poa pratensis*. Also present are a native grass, *Andropogon virginicus* and several species of forbs. Surrounding forested land supports *Ulmus* spp., *Quercus* spp., and *Juniperus virginiana*.

In March 2000, a Latin square, split-plot design experiment was established with fertilizer as the main plot factor and haying as the split plot factor. Main plots were 10 × 20 m, buffered by 2 m, and arranged in four, north–south oriented blocks. Sub-plots (hayed and not hayed) were 10 × 10 m. A total of 32 sub-plots represented fertilized (F), hayed (H), fertilized and hayed (FH), and control treatments. Fertilizer and haying treatments were applied according to regionally common management practices. Fertilizer was applied as ''N–P–K'' (N:P:K ratio of 29:3:4). Fertilization occurred as two equal doses totaling 16 g N m^{-2} in April and May 2000 and April and June 2001 and 2002, and in one dose of 14 g N m^{-2} in April 2003 and 2004. Haying occurred annually in June in 2001 and 2002, and in July 2003 and 2004.

Soil Sampling and Processing

In June 2004, we sampled mineral soil (0–15 cm, 5 cm diameter cores) at three random locations in each of the 32 plots. Samples were composited by plot, returned to the laboratory at the University of Kansas, and stored at 4°C until further processing. Within 3 days, soils were composited by management type to generate four sample types (F, H, FH, and control), and plant litter and all roots larger than 1 mm were picked from the soils via forceps. Field moist sub-samples were weighed, dried at 105°C for more than 48 h, and weighed again to determine gravimetric moisture content. Additional sub-samples of each soil type were subjected to aggregate size fractionation, acid hydrolysis, and long-term incubations.

Three replicate sub-samples of each soil type (total of 12 samples) were subjected to aggregate size fractionation (Accoe and others 2002). Sixty milliliters of deionized water was added to 20 g (fresh weight) of each sample and agitated vigorously. The slurries were wet-sieved on a shaker (New Brunswick Scientific, Edison, New Jersey) through a series of sieves (2,000, 212, 63 μm) into a receiving pan for 30 min. To ensure complete separation of the three size fractions, each fraction was washed and decanted onto the sieve(s) ten times.

Three replicate sub-samples of each soil type were also subjected to acid hydrolysis (Leavitt and others 1996). Ten grams of (fresh weight) samples was immersed in NaCl (1.2 g cm^{-3}) and mixed. Floating material was siphoned out. Remaining soil was washed free of salt and dried. Soil was then examined under a 20 × microscope, and all visible plant parts were removed via forceps. Plant-free soil sub-samples (3 g) were then subjected to acid hydrolysis via boiling 6 N HCl (NF/FCC grade, Fisher Scientific) for 18 h. Filtering the cooled mixture generated hydrolyzable and non-hydrolyzable fractions. The non-hydrolyzable fractions were washed; the hydrolyzable fractions were gently heated to evaporate remaining liquid. Both fractions were scraped into glass beakers for drying.

All soil fractions from aggregate size fractionations, acid hydrolysis, and bulk soil were dried at 70°C for more than 72 h, pulverized with a mortar and pestle, and weighed for total C and total N. Size fractions and dried, pulverized bulk soil samples were also analyzed for $\delta^{13}C$ and $\delta^{15}N$. Such data are useful for assessing the degree of coupling between SOM pools (Compton and Boone 2002). All samples were analyzed on a Carlo Erba elemental analyzer (1110 CHN Combustion Analyzer, Carlo Erba Strumentazione, Milan, Italy) coupled to a ThermoFinnigan Delta$^+$ mass spectrometer (Finnigan MAT, Germany). Precision of this instrument is ± 0.13‰ for $\delta^{13}C$ and ± 0.21‰ for $\delta^{15}N$.

Soils were also subjected to long-term incubations. Over 215 days, we measured microbial respiration and net N mineralization of three replicates of each soil composite, modifying the method suggested by Nadelhoffer (1990). Fifty g of field moist soil were weighed into PVC cores 5.5 cm in diameter and 7.5 cm in height, contained by glass fiber filter paper. On day 0, soils were leached with a N-free nutrient solution to remove inorganic N. Nutrient solution followed Nadelhoffer (1990). Soil cores were placed into air-tight jars lined with glass beads to permit air circulation beneath the filter paper. Jar lids were equipped with septa for periodic gas sampling. After sealing the incubation jars, gas samples were immediately taken and stored in previously evacuated, air-tight vials (Teledyne-Tekmar, Mason, OH, USA). On each sampling date, we extracted 10 ml gas samples from each incubation jar and injected them into air-tight vials. Soil containers were then removed and leached with N-free nutrient solution. Leachate was stored at 4°C until analysis for inorganic N content. Sampling occurred on days 1, 2, 3, 6, 10, 16, 21, 43, 70, 100, 126, 140, 161, 185, and 215.

Gas samples were analyzed for CO_2 concentration on a gas chromatograph equipped with a thermal conductivity detector (Varian, Walnut Creek, CA, USA). Rates of microbial respiration were calculated as the increase in CO_2-C evolution over time. Leachate samples were analyzed colorimetrically on a Lachat autoanalyzer (Madison, WI, USA). Net N mineralization was calculated as the sum of NH_4^+–N and NO_3^-–N extracted on each

sampling date. Rates of N mineralization and microbial respiration were calculated using the dry weight of the field moist soil and the incubation jar headspace.

We estimated pool sizes of organic C and N and their turnover rates using

$$C_t = C_l\left(1 - e^{-ht}\right) + (C_T - C_l)\left(1 - e^{-kt}\right) \quad (1)$$

where C_t is the cumulative amount of C respired at time t obtained from the incubation data, C_l is the model estimate of the labile C pool size, h is the estimated mineralization rate of that pool, C_T is total soil organic C obtained from elemental analyzer output, and k is the estimated turnover rate of the recalcitrant organic C pool (Andrén and Paustian 1987; Updegraff and others 1995; Bridgham and others 1998). A similar model was employed to assess labile and recalcitrant pools and turnover rates of organic N:

$$N_t = N_l\left(1 - e^{-ht}\right) + (N_T - N_l)\left(1 - e^{-kt}\right) \quad (2)$$

We estimated parameter values using a non-linear curve fitting procedure (PROC NLIN, SAS 8.02) on cumulative C and net N mineralization data. This procedure finds the best fitting equation by minimizing the sum of squares of the residuals. We tested the robustness of parameter estimates by changing the starting values of the iterative procedure to the maximum and minimum values specified by 95% confidence intervals (Billings and others 2004); no change in parameter estimates resulted.

Litterfall and Root Biomass Sampling

In the summer of 2004 (prior to haying), two strips in each subplot were sampled for litterfall. Each strip was 2×0.08 m wide (0.16 m^2). Litterfall from the two sampling strips in each subplot were merged to generate 32 samples. These litterfall samples were oven-dried to a constant weight (70°C), ground coarsely in a Wiley mill (Thomas Scientific, Swedesboro, NJ), and sub-samples were then finely ground using a mortar and pestle. For fine root analyses, all visible roots (< 1 mm in diameter) were removed from 200 g fresh weight of each soil composite described above. Roots were dried at 60°C for 24 h and weighed. These samples were then pulverized via mortar and pestle. We used previously published soil bulk density values for this site (Murphy 2004) to calculate root biomass per square meter, for the top 15 cm of the soil profile. Previous work indicates no significant trend of altered bulk density with plot treatment, though there was a trend of F and FH soils having lower values than control and H soils (difference of 0.05 g cm^{-3}, $P = 0.16$). Litterfall and root biomass were analyzed for C and N concentration and $\delta^{13}C$ and $\delta^{15}N$ as described for soils.

Litterfall was also subjected to cellulose and lignin analyses on an Ankom fiber analyzer (Ankom Technology, Fairport, NY, USA) (Billings and others 2003). Weighed sub-samples were analyzed for cellulose via heating and mixing with acid detergent (1N H_2SO_4 and cetyl trimethylammonium bromide). Samples were rinsed, dried via acetone evaporation and oven-drying (70°C, >24 h), and re-weighed. Samples were then analyzed for lignin content by agitating in 72% H_2SO_4, rinsing, drying via acetone evaporation and oven-drying, and reweighing.

Statistical Analyses

One-way analysis of variance (ANOVA; PROC GLM, SAS 8.02) was used to determine the effect of management technique (F, H, FH, control) on soil C and N concentrations and stable isotopic signatures. Plot treatment was considered a categorical variable for these analyses. Analysis of variance was also used to assess these effects on litterfall cellulose and lignin content. The Waller–Duncan K ratio t test was used as a post hoc test. We used a repeated measures, mixed random and fixed effects analysis (PROC MIXED, SAS 8.02) to assess the effect of management technique, sampling date, and their interaction on microbial respiration and net N mineralization. Differences in parameter estimates generated by the non-linear curve fitting procedure for incubation data were assessed using ANOVA (PROC GLM, SAS 8.02) with plot treatment as a categorical variable. Data were $\log_e$ transformed to generate a normal distribution when necessary. Statistical significance was determined at $\alpha = 0.05$. Errors are presented as one standard error of the mean.

Results

Bulk soil C concentrations were higher in F soils than in all other treatments (Figure 1A, 19.0 ± 0.2 vs. 17.0 ± 0.2 mg g_{soil}^{-1}). Nitrogen concentrations were higher in F soils (Figure 1B, 1.9 ± 0.0 mg g_{soil}^{-1}) than all other treatments, and bulk FH soils had higher N concentrations than control and H soils (both 1.3 ± 0.0 mg g_{soil}^{-1}). Bulk soil $\delta^{13}C$ and $\delta^{15}N$ values did not exhibit any significant differences between treatments (data not shown).

Soils from F treatment plots exhibited significant differences between size fractions in C concentration (Figure 1C); the largest size fraction (212–

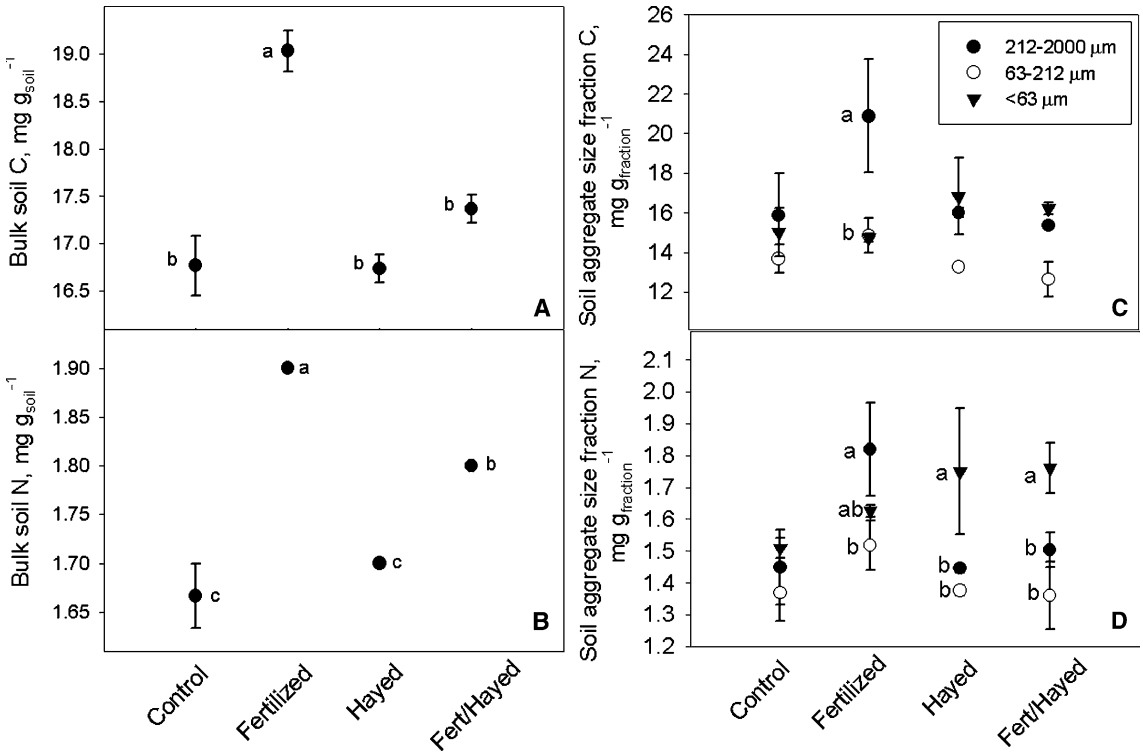

Figure 1. Mean concentrations of carbon (**A**) and nitrogen (**B**) in bulk soils and in aggregate size fractions (**C** carbon) and (**D** nitrogen) from control, fertilized, hayed, and fertilized/hayed plots. *Lowercase letters* indicate statistical significance ($\alpha = 0.05$) between treatments in (**A**) and (**B**), and within treatments in (**C**) and (**D**). Those treatments in (**C**) and (**D**) with no letters exhibited no significant differences between size fractions. *Error bars* represent one standard error of the mean.

2,000 μm) contained greater concentrations of C than the mid- (63–212 μm) and smallest (<63 μm) size fractions (20.9 ± 2.9 vs. 14.8 ± 0.6 mg $g_{fraction}^{-1}$). On a per g of bulk soil basis, these concentrations result in F soils' largest fraction being significantly greater in C concentration than all other treatments (9.31 ± 0.03 vs. 7.09 ± 0.39 mg $C_{212–2000}$ g_{soil}^{-1}). No other soil treatment elicited differences in C concentration among size fractions. All soil treatments except control soils exhibited differences in N concentration among size fractions (Figure 1D). In F soils, the largest size fraction contained significantly more N than the mid-sized fraction (1.8 ± 0.1 vs. 1.5 ± 0.1 mg $g_{fraction}^{-1}$). In H and FH soils, the smallest size fraction contained significantly more N than the larger two fractions (1.8 ± 0.2 vs. 1.4 ± 0.2 mg $g_{fraction}^{-1}$ for H, and 1.8 ± 0.1 vs. 1.5 ± 0.1 mg $g_{fraction}^{-1}$ for FH). The largest size fraction of control soils comprised a significantly smaller percentage of bulk soil than all other treatments (32 ± 4 vs. 50 ± 3%, respectively), and the smallest size fraction of control soils comprised a larger percentage of bulk soil than all other treatments (41 ± 4 vs. 23 ± 2%, respectively).

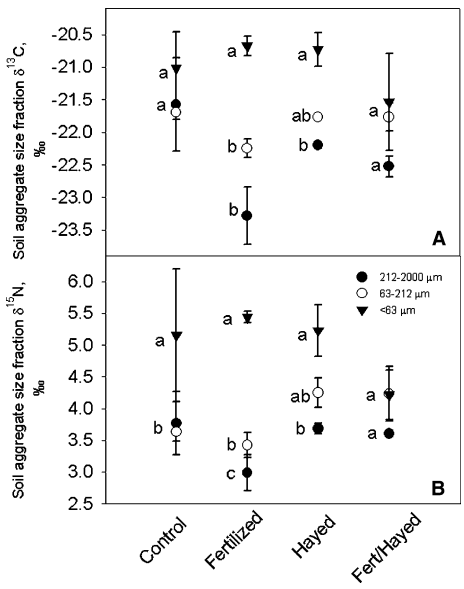

Figure 2. Mean $\delta^{13}C$ (**A**) and $\delta^{15}N$ (**B**) values of aggregate size fractions from control, fertilized, hayed, and fertilized/hayed plots. *Lowercase letters* represent statistical significance ($\alpha = 0.05$) within each treatment. *Error bars* represent one standard error of the mean.

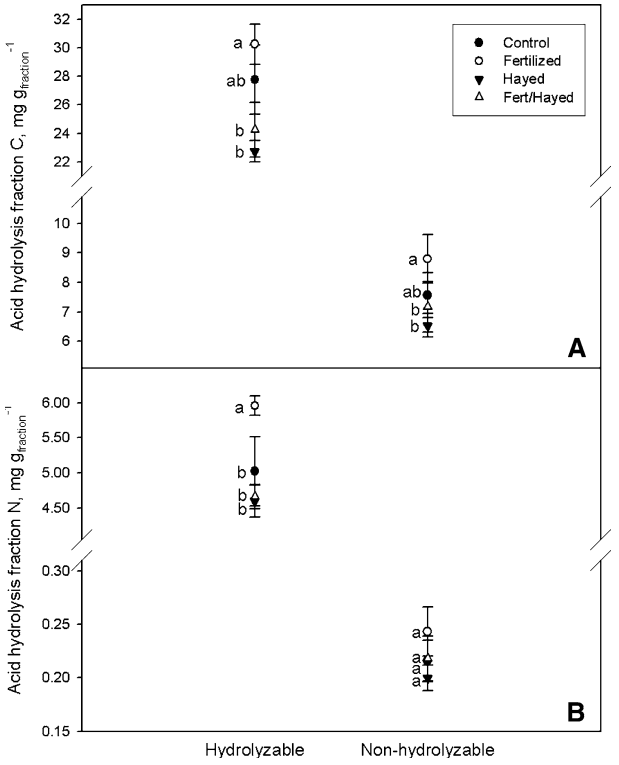

Figure 3. Mean carbon (**A**) and nitrogen (**B**) concentrations of hydrolyzable and non-hydrolyzable fractions of soils from control, fertilized, hayed, and fertilized/hayed plots. *Lowercase letters* represent statistical significance between treatments, within each fraction ($\alpha = 0.05$). *Error bars* represent one standard error of the mean.

Differences in $\delta^{13}C$ between size fractions were greatest in F soils (Figure 2A). The smallest size fraction possessed the most enriched $\delta^{13}C$ values (−20.7 ± 0.2‰), likely reflecting C fixed by native C4 vegetation prior to European settlement. The mid-sized fraction exhibited a non-significant trend of ^{13}C enrichment relative to the largest size fraction (−22.2 ± 0.1 vs. −23.3 ± 0.4‰, $P = 0.06$). Similar but less prominent differences existed in the H soils. Fertilization and H treatment soils induced a similar pattern of distinct $\delta^{15}N$ values among size fractions (Figure 2B). The smallest size fraction had the most enriched values (5.4 ± 0.1‰ for F, 5.2 ± 0.4‰ for H), and the largest size fraction exhibited relatively depleted values (3.0 ± 0.3‰ for F, 3.7 ± 0.1‰ for H). The smallest fraction in control soils was also enriched in ^{15}N relative to the two larger size fractions, but this difference (approximately 1.3‰) was smaller than in F soils. Fertilizer possessed $\delta^{15}N$ values of −0.2 ± 0.1‰.

All hydrolyzable fractions possessed greater C concentrations than non-hydrolyzable fractions (26.2 ± 1.7 vs. 7.5 ± 0.5 mg $g_{fraction}^{-1}$, Figure 3A). Both hydrolyzable and non-hydrolyzable fractions of F soils had higher C concentrations than these fractions in FH and H soils. No difference existed between these fractions in F soils and control soils. No significant differences existed between treatments for C concentration as expressed on a per g bulk soil basis in hydrolyzable or non-hydrolyzable fractions. There was a significant interaction between soil fraction and treatment for N content, with the hydrolyzable fraction of F soils possessing greater N concentrations (6.0 ± 0.1 mg $g_{fraction}^{-1}$) than the hydrolyzable fraction of all other soils (4.76 ± 0.13 mg $g_{fraction}^{-1}$, Figure 3B).

Non-linear curve fitting of incubation data generated labile C pool estimates that were 77% greater in F soils than in control soils (8.25 ± 0.32 vs. 4.65 ± 0.42 mg g_{soil}^{-1} for F and control soils, respectively). No significant differences in estimates of turnover rates of the labile (mean of 65.5 ± 7.2 μg C d^{-1} for all treatments) or recalcitrant (mean of 2.9 ± 0.3 μg C d^{-1} for all treatments) C pools were observed between treatments. Model estimates of organic N pool sizes and turnover rates exhibited no differences with treatment. Fertilized soils exhibited a non-significant trend of generating more CO_2-C than control soils in incubations (Figure 4A, $P = 0.14$ to 0.16 from day 43 through day 161). By the end of the incubation, all soils had respired a mean of 11.1 ± 0.7 mg C g_{soil}^{-1}. By day 21 of the incubation, F soils had experienced significantly more net N mineralization than control and H soils (Figure 4B, 5.98 ± 1.23 vs. 3.68 ± 0.21 μg N g_{soil}^{-1}). This trend remained statistically significant through day 70. By day 43 and day 70, FH soils had mineralized a mean of 192% of the N mineralized by control and H soils ($P < 0.05$). By the end of the incubation, all soils had mineralized a mean of 18.8 ± 1.2 μg N g_{soil}^{-1}.

Root biomass had higher concentrations of N in F and FH plots than in control and H plots (7.04 ± 0.12 vs. 5.71 ± 0.13 mg g_{root}^{-1}), and control plots had higher C concentrations than all other plots (276.69 ± 2.26 vs. 220.77 ± 3.85 mg g_{root}^{-1}). No biological (>1‰) differences were found among plots' root biomass samples for $\delta^{13}C$. Root $\delta^{15}N$ values for F and FH plots were higher than control and H plots by approximately 1.1‰. Aboveground plant litter N concentrations were higher in F and FH plots than in control and H (12.30 ± 0.51 vs. 8.44 ± 0.42 mg g_{litter}^{-1}), and C concentrations were higher in F plots than in all others (430.90 ± 5.50 vs. 394.90 ± 4.40 mg g_{litter}^{-1}). Plant litter $\delta^{15}N$ values were lowest in control and H plots (−3.8 ± 0.2‰) and highest in F

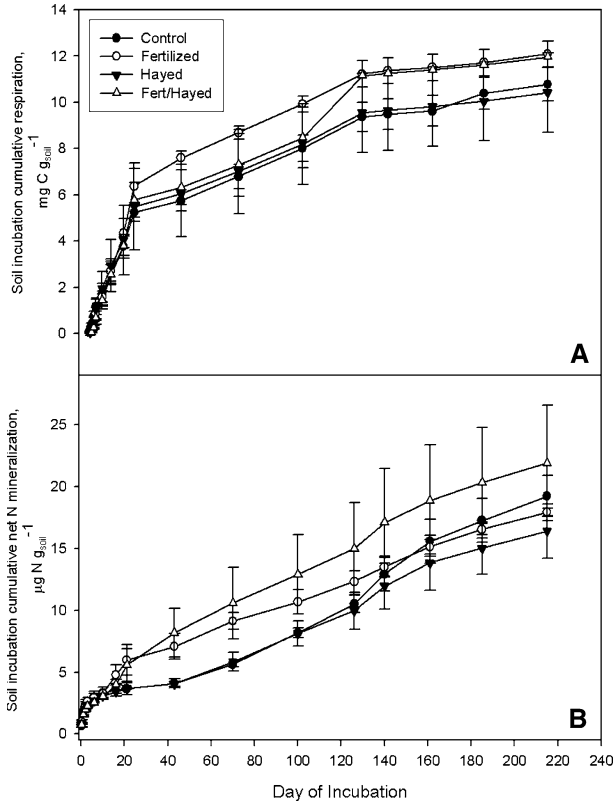

Figure 4. Mean cumulative carbon respired (**A**) and net nitrogen mineralized (**B**) during 215-day incubations of soil from control, fertilized, hayed, and fertilized/hayed plots. *Error bars* represent one standard error of the mean.

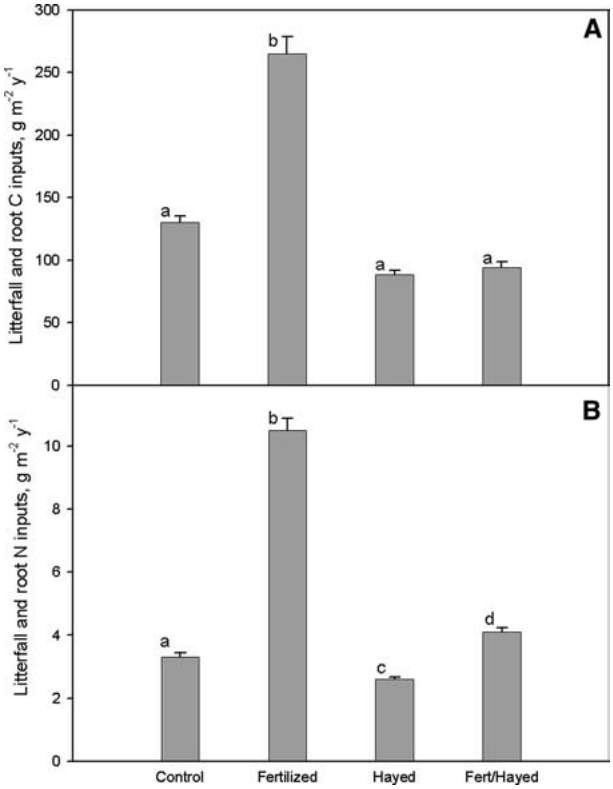

Figure 5. Mean carbon (**A**) and nitrogen (**B**) inputs to soil organic matter pools via litterfall and root biomass in control, fertilized, hayed, and fertilized/hayed plots. *Lowercase letters* represent statistical significance between treatments ($\alpha = 0.05$). *Error bars* represent one standard error of the mean.

and FH plots (−1.8 ± 0.2‰), reflecting the addition of fertilizer N (mean $\delta^{15}N$ value of −0.2 ± 0.1‰). Plant litter $\delta^{13}C$ values were lowest in F and FH plots (−27.7 ± 0.1‰) and highest in control and H plots (−24.6 ± 1.1‰), likely reflecting improved water status with fertilization.

Litterfall from the FH plots possessed greater concentrations of lignin than litterfall from control and F plots (293.0 ± 999.3 vs. 216.1 ± 14.4 mg g_{litter}^{-1}). No differences in cellulose content existed between plot treatments. Because litterfall collections across 3 years (2002–2004) indicate no increase in litterfall biomass with any treatment (B. L. Foster, unpublished data), we assume that litterfall data approximates yearly aboveground production, which permits calculations of yearly C and N inputs to the soil system when used in conjunction with root biomass data. Fertilized plots generated significantly more C and N inputs to the soil system via litterfall and roots than any other treatment (265.2 ± 13.7 and 10.5 ± 0.4 g m^{-2} y^{-1} for C and N, respectively, Figure 5A, B). These inputs represent a 104% increase over C inputs in control soils, and a 216% increase over litterfall and root N inputs in control soils. Hayed and FH soils experienced a 29% decline in C inputs via litterfall and root biomass, and a 22% decline in N inputs via litterfall and roots, relative to control soils.

The C:N ratios of litterfall and root biomass, weighted according to the contribution of each of these components to soil organic inputs, were greatest in control plots (47.49 ± 0.72), and lowest in FH plots (31.45 ± 0.99; Figure 6A). Parallel differences in size fractions of the mineral soil were not observed (Figure 6B). Fertilized and FH soils' largest size fractions had significantly greater C:N ratios than these soils' smaller size fractions.

Discussion

After 5 years, only F treatment plots experienced a significant (13.5%) increase in bulk soil C in the top 15 cm compared to control soils. Using a bulk density of 0.6 g cm^{-3} (Murphy 2004), this indicates that the top 15 cm of F soils increased in soil C content by approximately 360 kg C ha^{-1} y^{-1}. This is

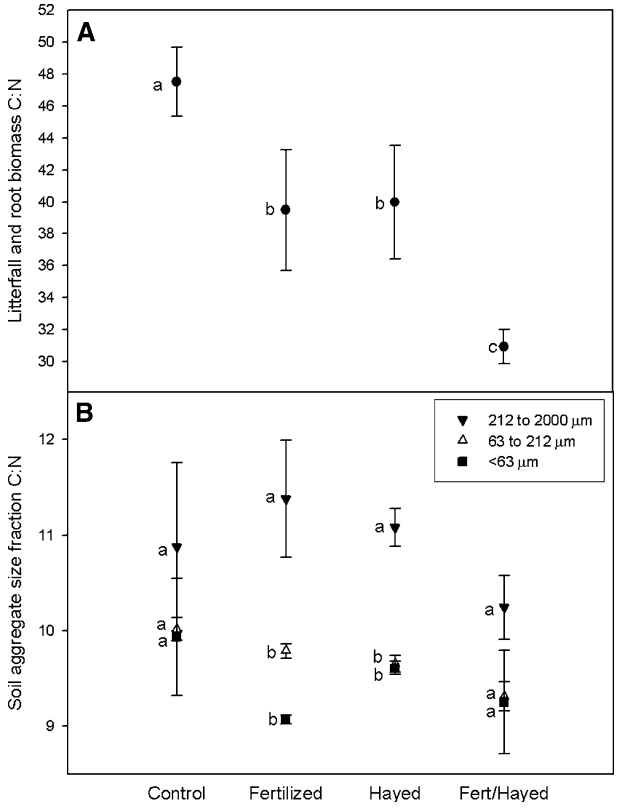

Figure 6. Mean C:N ratios of litterfall and root biomass (**A**) and soil size fractions (**B**) in control, fertilized, hayed, and fertilized/hayed plots. Mean ratios in (**A**) represent merged values of weighted C:N ratios for both litterfall and root biomass, according to the proportion of total organic inputs represented by each component. *Lowercase letters* in (**A**) represent statistical significance ($\alpha = 0.05$) between treatments. *Lowercase letters* in (**B**) represent statistical significance ($\alpha = 0.05$) between fractions, within each treatment. *Error bars* represent one standard error of the mean.

slightly more than the 200 kg C ha^{-1} y^{-1} estimated for the top 10 cm of soils after 27 years of annual fertilizer application to brome fields in Canada (Malhi and others 2003). Our estimate is also larger than the soil C increases reported for the top 15 cm of soil in agricultural systems receiving similar N inputs for 11 years (182 kg C ha^{-1} y^{-1}, calculated from Halvorson and others 1999). Our estimate likely is higher than these studies because we assessed soils after the first five years of treatment, and rates of soil C sequestration typically are highest during initial years of management practices intended to promote soil C storage (Huggins and others 1998).

Though there are some important exceptions to this assumption, the largest size fraction of SOM primarily harbors relatively labile, recently produced material (Tiessen and Stewart 1983; Balesdent and Mariotti 1996). Accoe and others (2004) indicate that the heaviest density fraction (>1.37 g cm^{-3}) of relatively large SOM size fractions (150–2,000 μm) can represent recently formed SOM. We did not incorporate both density and size fractionation techniques into this study, but size fractionation data presented here suggest that little of the C sequestered in F soils is incorporated into the two smaller fractions. The distribution of the percentage of total soil C was altered in F soils relative to controls. The increase in soil C exhibited in bulk F soils resulted from additional C harbored in the largest size fraction (212–2,000 μm), consistent with recently formed SOM being incorporated into more labile fractions. We can use soil organic N data as another indicator of SOM flows in these soils; total soil N data are generally consistent with the C data. Bulk F soils possessed higher organic N content, largely due to its incorporation into the largest size fraction. Data from acid hydrolysis fractions are also congruent with these results. The hydrolyzable fraction of F soils, which contains relatively labile material (Leavitt and others 1996), revealed additional organic N compared to all other soils.

Stable isotopic signatures of SOM and incoming plant material can help elucidate how dynamics into and between SOM pools have been altered with treatment. No statistically significant differences were found between treatments for bulk soil sample $\delta^{15}N$ values, and no differences were found for $\delta^{13}C$ that were biologically significant between bulk samples. However, fertilization promoted divergence among $\delta^{13}C$ and $\delta^{15}N$ values between size fractions, suggesting development of SOM pools that are relatively decoupled from each other (Compton and Boone 2002). This trend was particularly prominent in F soils. The smallest size fractions exhibited the highest $\delta^{13}C$ and $\delta^{15}N$ values, consistent with these fractions representing generally older material. For N isotopes in particular, relatively higher values can indicate a greater degree of microbial and chemical processing (Nadelhoffer and Fry 1994), which would occur if these fractions represent older material. If the largest size fraction represents primarily younger SOM (Tiessen and Stewart 1983; Balesdent and Mariotti 1996), such patterns suggest that the relatively young SOM augmented by fertilization is not readily incorporated into the older, smaller size fractions, and instead remains in SOM pools that are relatively easily metabolized by microbial activity (Balesdent and Mariotti 1996; Roscoe and others 2001; Accoe and others 2002). This is consistent with other fertilization studies in grasslands

that document increases in soil C in more readily mineralizable SOM fractions (Ludwig and others 2003; Malhi and others 2003).

Incoming plant material possessed lower $\delta^{13}C$ and $\delta^{15}N$ values than the soil that supported it, as described in numerous studies (Gebauer and others 1994; Nadelhoffer and Fry 1994; Buchmann and others 1997; Ehleringer and others 2000). The patterns observed in plant litter and root $\delta^{15}N$ values (F and FH > control and H) were not exhibited in $\delta^{15}N$ of SOM fractions, suggesting that fertilizer-derived N was lost from F and FH soils as plant material decomposed. Losses of NH_3 and N_2O in F and FH plots were significantly higher than in control or H plots during the 2005 growing season (S. A. Billings, unpublished data). This is congruent with other studies of N dynamics in fertilized systems (Bouwman and others 2002; Flynn and others 2005), but it is yet unclear what induced the smaller differences between soil and plant litter $\delta^{15}N$ in F and FH plots.

Parameter estimates modeled from incubation data and the mineralization rates from these incubations are consistent with soil C and N content and $\delta^{13}C$ and $\delta^{15}N$ data. Parameter estimates from the applied model (Updegraff and others 1995; Bridgham and others 1998) indicate an increase in the labile pool size for soil organic C with fertilization, contrasting with other fertilization studies that indicate increasing turnover rates of this pool (Dijkstra and others 2005). Our estimates of C content in hydrolyzable fractions (5.86 ± 0.39 $mg_{Chydr}\ g_{soil}^{-1}$) and the largest size fractions (9.31 ± 0.03 vs. 7.09 ± 0.39 mg $C_{212–2000}\ g_{soil}^{-1}$ for F and all other treatments, respectively) fell within the range of, or were close to, model estimates of labile pool sizes (8.25 ± 0.32 vs. 4.65 ± 0.42 mg C g_{soil}^{-1} for F and control soils), suggesting that these methods are useful for quantifying labile SOM fractions. Such results are valuable, given that estimates of soil C pools sometimes vary widely depending on the methods used (Paul and others 2003). Carbon mineralization rates from the incubations suggest that the soil C gains associated with fertilization in F soils may be readily available for microbial processing. The non-significant trends of increased soil respiration are important, given the following: (1) the conservative nature of the repeated measures test used to analyze the respiration data (Littell and others 1996); (2) these rates' correspondence with modeled parameter estimates; and (3) observed increases in net N mineralization during the incubation in these soils. Higher rates of net N mineralization in F soils correspond with other studies documenting the impact of fertilization on grassland organic matter processing (Rice and Garcia 1994).

Litterfall and root biomass in F plots increased by approximately 104 and 216% for C and N inputs, respectively, and were of higher quality as measured by C:N ratios. These increases in quality were not reflected in SOM size fractions (Figure 6B). This suggests that observed differences in quality of organic inputs were mitigated by microbial processing during the transformation of plant residues into SOM. Because the largest size fraction often represents relatively recently formed SOM (Tiessen and Stewart 1983; Roscoe and others 2001; Accoe and others 2002), much of the mitigation of quality differences may have taken place during initial transformations of plant residues into this size fraction. Such differences in organic matter processing suggest that increases in soil microbial population size, composition, and/or function have been promoted by fertilization (Fenn and others 1998; Schmidt and others 2004; Treseder 2004, Raiesi 2004). Such alterations could promote more effective microbial utilization of organic inputs with fertilization, which may help explain the lack of incorporation of additional C into more recalcitrant SOM fractions.

One means of promoting formation of stable SOM may be an increase in recalcitrant compounds in plant litter. Such processes have been observed in several systems, where fertilization has induced increased lignin content in plant residues (Miyagi 1983; Wolf and Opitz von Boberfeld 2003; Aerts and others 2003), though the opposite effect has also been reported (Lee and Lee 2000). Additional lignin production could add to the stabilization of SOM by increasing the amount of recalcitrant material being worked into the soil profile (Killham 1994). In this study, increases in lignin did not occur with fertilization; only in vegetation growing on FH soils did any increases in lignin concentration occur. These increases were not accompanied by any increases in total soil C or in soil C within the more stable SOM fractions, suggesting that increases in litterfall lignin may need to persist for more than 5 years to generate greater stability in SOM.

This study suggests that sequestering significant amounts of soil C may be a challenging goal using these management techniques in similar grasslands. Plots treated with haying or both fertilization and haying, treatments which accurately mimic management regimes in Midwestern North American grasslands, did not exhibit increases in soil C after 5 years of treatment. Fertilization alone, a management technique not commonly applied to grasslands without haying or grazing, did induce

significant increases in bulk soil C content, but contrary to many management goals, increases in soil C with fertilization did not result from C storage in the most stable soil fractions. After 5 years of treatment, increases in soil C in this experimental grassland appear to result from plant residues being processed into the largest soil fraction, which corresponds with model estimates of an increased labile C pool with fertilization. Significant portions of such C is likely to be mineralized within a relatively short timeframe (Huggins and others 1998), leaving only a fraction of C gains to be potentially stabilized into more recalcitrant pools. Soil C sequestration in managed grasslands may mitigate a portion of C emissions resulting from agricultural activities (Paustian and others 1997; Schlesinger 1999; Conant and others 2001), but this study suggests that long-term C gains representing a significant fraction of total fossil fuel C emissions may not be a realistic outcome of these management techniques in brome grasslands such as these at the prairie/forest ecotone.

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