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Grazing Impacts on Soil Carbon and Microbial Communities in a Mixed-Grass Ecosystem

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Good management of rangelands promotes C sequestration and reduces the likelihood of these ecosystems becoming net sources of CO_2. As part of an ongoing study, soil was sampled in 2003 to investigate the long-term effects of different livestock grazing treatments on soil organic carbon (SOC), total nitrogen (TN), and microbial communities. The three treatments studied (no grazing, EX; continuously, lightly grazed [10% utilization], CL; and continuously, heavily grazed [50% utilization], CH) have been imposed on a northern mixed-grass prairie near Cheyenne, WY, for 21 yr. In the 10 yr since treatments were last sampled in 1993, the study area has been subject to several years of drought. In the 0 to 60 cm depth there was little change in SOC in the EX or CL treatments between 1993 and 2003, whereas there was a 30% loss of SOC in the CH treatment. This loss is attributed to plant community changes (from a cool-season [C_3] to a warm-season [C_4] plant dominated community) resulting in organic C accumulating nearer the soil surface, making it more vulnerable to loss. Soil TN increased in the EX and CL treatments between 1993 and 2003, but declined in the CH treatment. Differences in plant community composition and subsequent changes in SOC and TN may have contributed to microbial biomass, respiration, and N-mineralization rates generally being greatest in CL and least in the CH treatment. Although no significant differences were observed in any specific microbial group based on concentrations of phospholipid fatty acid (PLFA) biomarkers, multivariate analysis of PLFA data revealed that microbial community structure differed among treatments. The CH grazing rate during a drought period altered plant community and microbial composition which subsequently impacted biogeochemical C and N cycles.

Abbreviations: AMF, arbuscular mycorrhizal fungi; CFI, chloroform-fumigation incubation; CH, continuously, heavily grazed; CL, continuously, lightly grazed; EX, ungrazed exclosure; HPGRS, High Plains Grasslands Research Station; MAP, mean annual precipitation; MB, microbial biomass; MR, microbial respiration; N_i, inorganic N; Nmin, N-mineralization; NPP, net primary production; PLFA, phospholipid fatty acid; SOC, soil organic carbon; SOM, soil organic matter; TN, total nitrogen.

Rangelands occupy nearly 50% of the world's land area and are estimated to contain more than one-third of the world's above- and belowground C reserves (Allen-Diaz, 1996). Soil organic C storage in rangeland soils is influenced by climate, biome type (Conant et al., 2001), management (grazing, N inputs, restoration) (Follett et al., 2001, Derner and Schuman, 2007), and environmental conditions (drought, climate change) (Jones and Donnelly, 2004). Carbon sequestration estimates on rangelands vary from 0.02 to 0.08 Mg C ha^{-1} yr^{-1} in arid climates, 0.03 to 0.12 Mg C ha^{-1} yr^{-1} in semiarid climates, and 0.08 to 0.20 Mg C ha^{-1} yr^{-1} for semi-humid to subhumid environments (Lal, 2000). Although the magnitude of SOC storage per unit area is small relative to other land uses, increased C storage on rangelands as a result of management can be significant considering the large land area they represent (Follett, 2001). For instance, Schuman et al. (2001) estimated that improved management of 113 Mha of poorly managed rangelands in the United States could sequester an additional 11 Tg of C annually relative to no change in management strategies. In addition, they estimated that the loss of 43 Tg C yr^{-1} could be avoided through the continued use of sustainable grazing practices, conservation of undisturbed, native rangelands, restoration of marginal croplands to perennial grasslands

through the Conservation Reserve Program (CRP) and ensuring CRP lands are not recropped (Schuman et al., 2001).

Livestock grazing occurs widely across the northern mixed-grass ecosystem (Kimble et al., 2001), however, ecosystem properties and processes such as plant community composition and structure, soil characteristics, and nutrient cycles can be affected by grazing management practices. Both the stocking rate and duration of livestock grazing can affect plant community composition through displacement of C_3 grasses by C_4 grasses in mixed-grass prairies (Dormaar and Willms, 1990). Grazing also influences the amount and composition of soil organic matter (SOM) (Dormaar and Willms, 1990; Frank et al., 1995) through its effects on litter accumulation and decomposition (Naeth et al., 1991; Shariff et al., 1994). The extent to which different grazing practices alter C cycling and SOM composition is not very well understood (Schuman et al., 1999; Parton et al., 2001; Ganjegunte et al., 2005). Lack of a clear relationship between grazing practices and SOC has been attributed to soil heterogeneity, inconsistent depth of soil sampling, and insufficient understanding of C and N distributions within the grazing system (Schuman et al., 1999). It is important that we have a clear understanding of grazing management effects on C accumulation so that effective soil C management plans can be developed for rangelands (Kaiser, 2000).

Research is needed to fully understand the effects of climate on C biogeochemistry of rangelands and to determine the main factors affecting the accumulation and long-term storage of SOC (Jones and Donnelly, 2004). Climatic factors such as precipitation (Schuman et al., 2005) and temperature (Bellamy et al., 2005) may have more pronounced affects on C accumulation and long-term storage than earlier believed. Derner and Schuman (2007) reviewed the available literature and found that soil C storage in the upper 10 cm of soil occurred when precipitation was <440 mm yr^{-1}, which is consistent with the findings of Sims et al. (1978) and Sala et al. (1988). Balogh et al. (2005) and Breshears and Allen (2002) suggested drought may induce losses of C from rangelands resulting in these ecosystems changing from net sinks to sources of C. Schuman et al. (1999) and Frank (2004) showed considerable C accumulation in mixed-grass rangeland soils during a period of average to above average precipitation, however, the long-term stability of SOC in grazed prairie ecosystems is unknown.

Soil microorganisms play a central role in C cycling, (e.g., decomposition, respiration, humification, physical stabilization) and, as such, will greatly influence the storage of C in SOM. The manner in which these processes influence SOM are important to N cycling, as N is usually the factor limiting productivity (after precipitation) in rangeland ecosystems (Krueger-Mangold et al., 2004). Changes in substrate availability (C or N) required for mineralization have the potential to influence not only microbial growth, but also plant communities (Wedin and Tilman, 1990). To better understand C and N cycling, it is important to gain an understanding of microbial communities and related processes and how they are affected by grazing.

The objective of this research was to evaluate the long-term (21 yr) influence of grazing on SOC and soil TN over the course of two different time periods (1982–1993 and 1993–2003) that differed greatly in rainfall (above and below average) on a northern mixed-grass prairie. Moreover, given the influential role that soil microorganisms have on C and N cycles, we also investigated how grazing affected microbial processes (N and C mineralization) and microbial communities (biomass and structure).

MATERIALS AND METHODS

Study Background

This study was part of an ongoing field experiment undertaken at the USDA-ARS High Plains Grasslands Research Station (HPGRS) near Cheyenne, WY, to investigate the long-term influence of different grazing rates on a northern mixed-grass prairie ecosystem. Stocking rate treatments were established in 1982 in a randomized block design with two replicate blocks (pastures) for each of the three stocking rates using a season-long grazing strategy (Hart et al., 1988). The three stocking rate treatments were: EX, ungrazed exclosures; CL, pastures grazed season-long at a light stocking rate of 0.16 to 0.23 steers ha^{-1}, and; CH, pastures grazed season-long at a heavy stocking rate of 0.56 steers ha^{-1}. The stocking rate of the CL treatment was about 35% below that recommended by the NRCS, whereas the CH treatment had a stocking rate 33% above that recommended by the NRCS. About 10% of the aboveground net primary production (NPP) was used in the CL treatment, whereas about 50% of NPP was used in the CH treatment (LeCain et al., 2000).

In 1993 the three grazing treatments were sampled for soil C and N as well as for above- and belowground vegetation production (Schuman et al., 1999). These sites were resampled in 2003 and it is these results we present in this paper. We would like the reader to note some differences in sampling and laboratory methods and, though they do not change our conclusions, they are worth noting. In 1993, soil cores were separated into 0- to 3.-8, 3.8- to 7.6-, 7.6- to 15-, 15- to 30-, 30- to 45-, 45- to 60-, and 60- to 90-cm increments, whereas in the current study soils were sampled at 0- to 5-, 5- to 15-, 15- to 30-, and 30- to 60-cm increments. We did not sample the 60- to 90-cm depth increment due to the hardness of the soil. In the original study, Schuman et al. (1999) reported above- and belowground plant biomass production. We elected not to sample belowground plant biomass for the following reasons: (i) soils were sampled in the first week of May and thus our estimates of root production would not have matched peak aboveground biomass production that occurs in early July; (ii) it is unlikely that above- or belowground production would have varied greatly from what was previously reported after allowing for precipitation differences, and (iii) the major objective of this study was to ascertain the influence of grazing management on soil C and N. Finally, different methods were used to determine soil C and N. In the original paper, organic C was determined by the Walkley–Black dichromate oxidation method and organic N by the micro-Kjeldahl procedure, whereas in the current study, a dry combustion method was used to determine both soil C and N concentrations. Previous work from the HPGRS Soil Laboratory (data not shown) using soils (n = 100) from different depths and with different concentrations of C and N found no significant difference in C and N concentrations between dry combustion and dichromate oxidation (C) or micro-Kjeldahl (N) methods.

Study Site

The research study was conducted at the HPGRS (41°11′ N, 104°53′ W) near Cheyenne, WY, on a native, northern mixed-grass rangeland that ranges in elevation from 1910 to 1950 m. Climate at

the site is semiarid, with an annual frost-free period of 127 d. Long-term mean annual precipitation (MAP) at the HPGRS is 425 mm, of which 73% occurs from 1 April through 30 September (HPGRS data, 1977–2005). Grazing treatments were located principally on Ascalon (fine-loamy, mixed, superactive, mesic Aridic Argiustolls) and Altvan (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Aridic Argiustolls) soils (Stevenson et al., 1984).

Vegetation on this northern mixed-grass prairie is dominated by grasses (55% C_3 species and 23% C_4 species), forbs, sedges, and half shrubs (Schuman et al., 1999). Dominant C_3 species are western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Löve] and needleandthread [*Hesperostipa comata* (Trin & Rupr.) Barkworth]. The principal C_4 species is blue grama [*Bouteloua gracilis* (Willd. Ex Kunth) Lag. ex Griffiths]. Legumes comprised <2% of the plant community of this northern mixed-grass prairie ecosystem (Schuman et al., 1999). Before the establishment of this grazing study in 1982, domestic livestock had not grazed the study sites for more than 40 yr.

Field Sampling

Before initiating the grazing study in 1982, a 50-m permanent transect was established in each pasture on nearly level sites. Soils were sampled at five points (0, 10, 20, 30, and 40 m) along each 50-m transect, in early May 2003. A trowel was used to sample the 0- to 5-cm soil depth and thereafter a hydraulic soil sampler (Giddings Machine Co., Windsor, CO) was used to sample the 5- to 15-, 15- to 30-, and 30- to 60-cm depth increments. Three cores (4 cm i.d.) were obtained at each sampling point and composited to ensure adequate soil material was available for analyses. Separate soil cores were collected at the 10- and 30-m points along each transect to determine soil bulk density. Bulk density data were used to convert SOC and N concentrations (g kg^{-1} soil) to mass (Mg ha^{-1}). As soils contained <1% fine gravel, no adjustment of bulk density was necessary (Blake and Hartge, 1986). Soil samples were air-dried and sieved (2 mm) to remove plant crowns, visible roots, and root fragments and then stored at room temperature until analyzed.

Approximately 2 wk after soils were sampled for C and N, soils from each of the three treatments were sampled at five points (0, 10, 20, 30, and 40 m) along the same permanent transects for PLFA. Soils were sampled for the 0 to 5 cm increment using a trowel and at the 5 to 15 cm depth increment using a soil step probe (3 cm i.d., AMS Inc., American Falls, ID). Soils were placed into plastic bags and placed on dry ice until they could be stored at – 20°C (<4 h).

In 2002, a season long (6 May–30 July) in situ N-mineralization experiment was undertaken on the grazing treatments to investigate the influence that changes in plant community composition may have had on N cycling. At 10-m intervals along the same 50-m permanent transect within each of the replicate treatments, a perforated polyvinylchloride (PVC) core (5 cm i.d., 15-cm length) (Adams and Attiwill, 1986) was inserted 10 cm into the ground. Because of the relatively homogenous nature of the mixed-grass prairie ecosystem, no attempt was made to stratify cores around vegetation, and soils were sampled randomly. Soil within the core (T_0) was removed and placed into a plastic bag and stored in a cooler. The PVC core was then reinserted near where the T_0 sample had been taken and allowed to incubate for a period of time (T_1). If the T_0 soil sample had been taken from an area of bare soil or located in a patch of grass, the T_1 sample was also placed in a comparable area. Approximately 2 to 3 wk later, the PVC core was removed, and the soil within the core (T_1 soil) placed in a plastic bag and stored in a cooler. This process was repeated five times over a period of 12 wk (incubation periods varied from 11–22 d). After collection (<2 h), all soil samples were taken back to the laboratory and stored at 4°C until they could be analyzed.

Vegetation for this long-term grazing study was sampled annually in late July/early August (depending on precipitation and growth for that year). In each replicate pasture, six 0.18 m^2 quadrats were clipped to ground level and plant species present in each quadrat were sorted into functional groups (C_3 perennial grasses, C_4 perennial grasses, sub-shrubs, perennial forbs, annual forbs as well as standing dead material and litter). Each quadrat was protected from grazing during the growing season by temporary 1 m^2 exclosures that were moved a random distance and cardinal direction each year before grazing. Current growing season production was separated from older biomass, dried at 60°C, and weighed (Derner and Hart, 2007).

Laboratory Analyses

From the soil samples that had previously been air-dried and sieved (2 mm), a 100-g subsample was air elutriated to remove the remainder of the fine root material. A subsample of this material was roller-ground overnight to a fine powder (90% of material passed through a 0.25-mm sieve) on which inorganic and total C and TN were determined. Total C and N were determined using a C/N analyzer (NA2100 Protein, Carlo-Erba Instruments, Italy). Inorganic C was determined using a modified pressure-calcimeter method (Sherrod et al., 2002), and organic C was calculated by subtracting inorganic C from total C. All C and N values are expressed on an oven-dried basis (55°C).

Subsamples of air-dried and sieved soils from the 0- to 5- and 5- to 15-cm depths were analyzed for microbial biomass (MB), potential microbial respiration (MR), and potential N-mineralization (Nmin). All determinations were undertaken on samples that were rewetted to a soil–water content of –0.05 MPa, resulting in about 50% water-filled pore space. The measurement of M_R was undertaken on 50 g of air-dried soil using standard base trap methods (Zibilske, 1994) over a 21-d period. Potential N-mineralization was determined on the same soil on which M_R was measured by calculating the difference in soil inorganic N (N_i; NO_3 + NH_4) between Day 21 and Day 0. Potential nitrification was determined by calculating the difference in NO_3 between Day 21 and Day 0. Inorganic N was determined by extracting 5 g of soil with 50 mL of 1 *M* KCl, filtering through a Whatman no. 40 filter paper, and then analyzing extracts on a TRAACS 800 Auto-Analyzer (Technicon Industrial Systems, New York). A second 5-g subsample was dried at 105°C for 24 h to determine soil moisture content. Soil MB was estimated on 50 g of soil using the chloroform-fumigation incubation (CFI) method (Horwath and Paul, 1994) using a K_c of 0.41 (K_c is the fraction of MB-C decomposed and released as CO_2–C in 10 d; Anderson and Domsch, 1978). While use of air-dried soil will give rise to a flush of mineralization, we believe that it would have limited influence on our results as it typically occurs for less than a week (Franzluebbers et al., 2000). Moreover, as all soils were subjected to the same treatment, that for comparative purposes, this flush would not greatly influence any underlying differences among treatments.

Soils collected from the in situ N-mineralization experiment were sieved to 2 mm and two 5-g subsamples were taken to determine N_i and soil moisture as described above. Nitrogen mineralization was calculated as the difference between N_i at T_1 and T_0. Plant uptake of N_i was measured separately by calculating the difference between N_i at T_1 and T_0 from the following incubation period.

Soil samples for PLFA analysis were sent frozen to the USDA-ARS Sustainable Agricultural Systems Laboratory, Beltsville, MD, where they were analyzed for PLFA's using a modified Bligh and Dyer methodology (Frostegård and Bååth, 1991). Qualitative and quantitative fatty acid analysis was performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) and Sherlock software (MIDI Inc., Newark, NJ). The fatty acids used as biomarkers to represent eubacteria were 15:0, i15:0, i17:0, a17:0, cy17:0, and cy19:0; gram- positive bacteria; iso and anteiso-branched chain fatty acids; gram-negative bacteria, monounsaturated fatty acids; fungi, 18:2ω6c; arbuscular mycorrhizal fungi (AMF) 16:1ω5; actinomycetes, 10-Me fatty acids; protozoans, 20:3 and 20:4 (Buyer et al. (1999) and references therein, Allison et al., 2005). The nomenclature used to describe fatty acids is X:YωZ where X is the total number of C atoms in the chain, Y is the number of double bonds, and Z is the position of the double bond from the methyl end of the chain. The prefixes i, a, and Me indicate iso, anteiso, and midchain methyl branching with cy indicating a cyclopropyl ring structure.

Statistical Analysis

Analysis of variance was used to evaluate treatment effects on SOC and N using a randomized complete block design with two replicate blocks with 'treatment' and 'depth' as the main factors. The mean of the five sampling points along a transect for a given depth was calculated and this was used as a 'replicate' value (two replicates per treatment) for each soil parameter of interest. Analysis of variance was undertaken using the PROC GLM procedure (SAS ver. 9.1, SAS Institute, Inc., Cary, NC). There were no treatment × depth interactions. Least significant differences at $P \leq 0.10$ were used to separate treatment means when there was a significant treatment effect (Steel and Torrie, 1980) using the LSMEANS procedure. Comparison between the 1993 and 2003 SOC and TN data were analyzed as a repeated measures ANOVA (SAS Institute, Inc., Cary, NC).

For the microbial, PLFA, and N-mineralization (field and laboratory) data, ANOVA's (SuperANOVA, Ver.1.11, SAS Institute, Inc., Cary, NC) were undertaken using the same experimental design noted above. When a significant treatment × depth interaction was observed, a one-way ANOVA with 'treatment' as the main factor was used for each soil depth interval to determine treatment differences using a $P \leq 0.10$. Phospholipid fatty acid biomarker data was analyzed using a one-way MANOVA with treatment as the main effect and biomarker groups as the response variables (Proc GLM, SAS Institute, Inc., Cary, NC). The two depths were analyzed separately. The results are presented using canonical variates analysis, which is simply a graphic presentation of the MANOVA results. This analysis produces linear combinations of variables (PLFA biomarker groups) that maximize separation of treatment means (Buyer et al., 1999). The Shannon diversity index was used to calculate a measure of microbial diversity using the specific PLFA microbial markers (Shannon and Weaver, 1949).

RESULTS AND DISCUSSION

General Soil Properties

Bulk density increased significantly with depth, but there were no differences across treatments for a given depth (data not shown). Across all treatments, mean bulk density in the 0 to 5 cm depth increment ranged from 1.04 to 1.10; 5 to 15 cm, 1.36 to 1.43; 15 to 30 cm, 1.36 to 1.52, and; 30 to 60 cm, 1.39 to 1.59 Mg m^{-3}. In 1993 bulk densities were 1.00 to 1.17 (0–7.5 cm), 1.31 to 1.44 (7.5–30 cm) and 1.26 to 1.47 Mg m^{-3} (30–60 cm) (Schuman et al., 1999). Allowing for the different depth intervals sampled in 1993 and 2003, there were few differences in bulk density between the two sampling times, and in most cases the differences between 1993 and 2003 were <10%. Soil pH was not measured in this study but Schuman et al. (1999) reported a range of 6.4 to 7.3 in 1993.

Soil Organic Carbon

In 2003, 21 yr after initiation of the grazing treatments and 10 yr after the 1993 assessment of SOC in this grazed ecosystem, significantly lower concentrations of SOC in the CH and EX treatments were found compared to the CL treatment at both the 0 to 5 and 15 to 30 cm depths (Table 1). This translated into a significant loss of SOC in the CH treatment in 2003 compared to the mass of SOC measured in 1993 in the 0- to 15-, 0- to 30-, and 0- to 60-cm soil depths (Table 2). At the other depth increments (5–15 and 30–60 cm), SOC was in the order of CL > EX > CH (Table 1).

In the 21 yr since inception of the grazing treatments, measurable changes in plant community composition and productivity have been recorded. In the CH treatment, C_3 plant production has declined by almost half relative to the EX and CL treatments (Table 3) and has become increasingly dominated by the C_4 grass, blue grama (Table 3). The C_3 grasses represent the main component of range productivity (approximately 55%) on the northern mixed-grass prairie and their growth cycle is closely tied to precipitation patterns of

Table 1. Concentration and mass of soil organic carbon (SOC) and total nitrogen (TN) for soils from exclosed (EX), continuously, lightly grazed (CL), and continuously, heavily grazed (CH) pastures sampled in 2003 at the High Plains Grassland Research Station (HPGRS), Cheyenne, WY.

	SOC						TN					
Soil	Concentration			Mass			Concentration			Mass		
depth	EX	CL	CH	EX	CL	CH	EX	CL	CH	EX	CL	CH
cm	g kg^{-1} soil			Mg ha^{-1}			g kg^{-1} soil			Mg ha^{-1}		
0–5	20.9[b]†	26.1[a]	19.8[b]	10.8[b]	13.8[a]	10.9[b]	1.83[b]	2.31[a]	1.71[b]	0.94[b]	1.23[a]	0.94[b]
5–15	11.6[a]	13.4[a]	10.8[a]	16.5[a]	18.1[a]	15.1[a]	1.15[a]	1.36[a]	1.08[a]	1.65[a]	1.83[a]	1.53[a]
15–30	8.8[b]	11.0[a]	7.7[b]	20.0[a]	22.2[a]	16.6[b]	0.93[a]	1.11[a]	0.84[a]	2.11[a]	2.25[a]	1.80[a]
30–60	7.8[a]	9.3[a]	5.9[a]	33.2[a]	38.3[a]	28.0[a]	0.81[a]	0.96[a]	0.62[a]	3.45[a]	3.96[a]	2.92[a]
0–15	–	–	–	27.3[b]	32.0[a]	26.0[b]	–	–	–	2.59[b]	3.06[a]	2.47[b]
0–30	–	–	–	47.3[b]	54.2[a]	42.5[b]	–	–	–	4.70[ab]	5.31[a]	4.27[b]
0–60	–	–	–	80.5[b]	92.5[a]	70.5[b]	–	–	–	8.15[ab]	9.27[a]	7.19[b]

† For a given parameter and depth, lowercase letters that are different from one another indicate a significant ($P \leq 0.10$) grazing treatment response.

the area, where 41% of the precipitation occurs in April, May, and June (Western Regional Climate Center, 2007). This shift in community composition resulted in a nearly 20% reduction in the 2004 peak aboveground plant production in the CH treatment (Table 3) relative to the other treatments. This reduction is similar in magnitude to the difference between treatments reported by Derner and Hart (2007) for the period 1991 to 2006. This reduction in aboveground biomass in the CH compared to the EX and CL treatments resulted in lower potential C inputs from both above- (Table 3) and belowground litter production (Schuman et al., 1999).

Table 2. Comparison of masses of soil organic carbon (SOC), total nitrogen (TN), and C to N ratios in exclosed (EX), continuously, lightly grazed (CL), and continuously, heavily grazed (CH) pastures at the High Plains Grasslands Research Station (HPGRS), Cheyenne, WY, between 1993 and 2003. Figures in parentheses indicate the percent change between the 1993 and 2003 sampling dates.

Parameter	Soil depth	1993†			2003		
		EX	CL	CH	EX	CL	CH
	cm						
SOC, Mg ha^{-1}	0–15	28.2^{bA}‡	35.1^{a}	36.0^{aA}§	27.3^{b} (–3.1)	32.0^{a} (-9.0)	26.0^{bB} (-27.8)
	0–30	47.9^{b}	58.0^{a}	58.3^{aA}	47.3^{b} (-1.3)	54.2^{a} (-6.5)	42.5^{bB} (-27.1)
	0–60	88.1^{b}	91.9^{b}	101.3^{aA}	80.5^{b} (-8.7)	92.5^{a} (+0.6)	70.5^{bB} (-30.4)
TN, Mg ha^{-1}	0–15	2.34^{b}	3.07^{a}	2.80^{a}	2.59^{b} (+10.5)	3.06^{a} (-0.5)	2.47^{b} (-12.0)
	0–30	4.28^{c}	5.52^{a}	4.81^{b}	4.70^{ab} (+9.8)	5.31^{a} (-3.8)	4.27^{b} (-11.2)
	0–60	7.65^{a}	8.34^{a}	7.76^{a}	8.15^{ab} (+6.4)	9.27^{a} (11.1)	7.19^{b} (-7.4)
C to N ratio	0–15	12.0	11.4	12.8	10.5	10.5	10.5
	0–30	11.2	10.5	12.1	10.1	10.2	10.0
	0–60	11.5	11.0	13.1	9.9	10.0	9.8

† From Schuman et al. (1999).

‡ Means within a soil depth and year with different lowercase letters are significantly different at $P \leq 0.10$.

§ Means within a soil depth and across years with different uppercase letters are significantly different at $P \leq 0.10$.

Root C inputs have also changed as the mix of C_3 and C_4 species are now different between the grazing treatments. Typically about 56% of western wheatgrass (C_3) root biomass occurs in the top 15 cm and about 86% in the 0- to 60-cm depth increment (Weaver and Darland, 1949). Needleandthread (C_3) roots are also found predominantly in the top 45 to 90 cm (Coupland and Johnson, 1965). In contrast, the bulk (83%) of blue grama (C_4) roots are found in the top 15 cm with the 0- to 30-cm depth increment containing approximately 92% of the total root biomass (Weaver and Darland, 1949, Coffin and Lauenroth, 1991). Near-surface root abundance allows blue grama plants to take advantage of small precipitation events that occur later in the growing season (Sala and Lauenroth, 1982). In addition, short-grass steppe plant species (dominated by blue grama) deposit a greater proportion of their C belowground (Coupland and Van Dyne, 1979). Moreover, under conditions of heavy grazing (>90% defoliation), Buwai and Trlica (1977) observed no change in either root weight or carbohydrate reserves in blue grama, whereas there was a significant decline in total carbohydrate reserves in western wheatgrass roots. Such results suggest that when defoliated, that instead of storing carbohydrates, western wheatgrass uses these sugars to produce new foliage (Painter and Detling, 1981). With changes in root distribution, C will be located closer to the soil surface where it is vulnerable to decomposition and oxidation, and eventual efflux to the atmosphere as CO_2 or CH_4. In addition, an increase (50–90%) in bare soil area in the CH relative to the CL and EX treatments (data not shown) may also contribute to a loss of SOC present in soils under the CH treatment via wind and, to a lesser extent, water erosion (Neff, 2005). Changes in the quality, quantity, and location of C would seem to be important factors in regulating C pools in grazed ecosystems.

In concert with changes in vegetation composition, recent research has reported that climate change, specifically, variations in temperature and precipitation, are partially responsible for significant losses of SOC (Bellamy et al., 2005). Soil temperature data collected from an ungrazed area near the study sites have exhibited a general increase during the study period of 1982 to 2003 at both the 38- and 102-mm soil depths (Fig. 1). This increase in soil temperature was reflected in both the yearly and April to July averages over the 21-yr study period. Why soil temperatures increased at such a linear rate when there was little increase in mean or maximum air temperatures (Fig. 1) is unknown. Low soil moisture conditions prevalent from 1994 to 2003, may have resulted in an overall reduction in evaporation and leading to an increase in soil temperature. A previous study by LeCain et al. (2000) on the same grazing plots hypothesized that grazing resulted in higher soil temperatures and this has been confirmed by other studies (Bremer et al., 1998, 2001). Increases in soil temperature and vapor pressure deficit (conditions that were both likely to have been prevalent during summer in this area) have been shown to result in a loss of C to the atmosphere (Sukyer and Verma, 2001), and may have contributed to greater rates of SOM decomposition.

As noted in the introduction, an important objective of this study was to compare changes in SOC during two periods in which precipitation differed. Mean annual precipitation at the HPGRS from 1982 to 1993 was 475 mm, 12% greater

Table 3. Aboveground biomass and proportion of total biomass for various functional groups present in exclosed (EX), continuously, lightly grazed (CL), and continuously, heavily grazed (CH) pastures at the High Plains Grasslands Research Station HPGRS, Cheyenne, WY in 2004.

Functional group	EX	CL	CH	EX	CL	CH
	kg ha^{-1}			%†		
C_3–Perennial grasses	706	764	321	62	64	33
C_4–Perennial grasses	46	128	404	4	11	42
Sub-shrubs	163	9	150	14	1	16
Perennial forbs	145	105	84	13	9	9
Annual forbs	78	182	1	7	15	0
Total live aboveground biomass	1138	1188	960	100	100	100
Standing dead	260	232	43			
Litter	1447	802	413			
Total aboveground biomass	2845	2222	1416			

† As a percentage of Total live aboveground biomass.

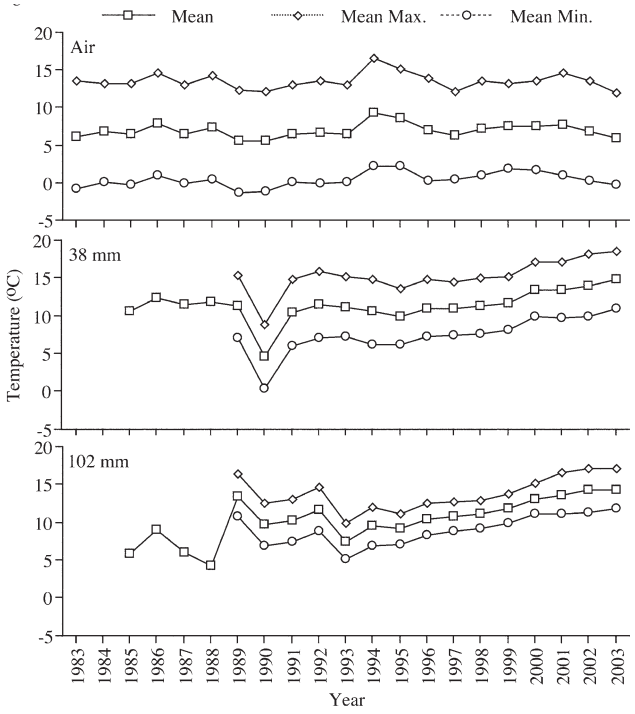

Fig. 1. Annual mean (Mean), maximum (Max.), and minimum (Min.) air and soil (38- and 102-mm depths) temperatures (1983–2003) at the High Plains Grasslands Research Station (HPGRS), Cheyenne, WY.

than the long-term MAP of 425 mm (1977–2005, HPGRS data). From 1994 to 2003, MAP was 339 mm (30% of normal) due to 7 yr of below-average rainfall, which included 5 yr (1994, 1998, 2000, 2001, and 2002) where only about 60% of the normal April to June precipitation and 67% of MAP occurred (Fig. 2). The flux of C from the soil to the atmosphere during periods of low rainfall and/or drought conditions in comparable ecosystems has been well documented (Meyers, 2001; Frank, 2004; Hunt et al., 2004). This loss of SOC is further compounded when prairies are heavily grazed. Morgan et al. (2004), at the Central Plains Experimental Range (40 km south of Cheyenne, WY), measured greater soil emission of CO_2 on heavily grazed (75% utilization) shortgrass steppe compared to ungrazed pastures during the same years of drought (2001 to 2003) as at the HPGRS. This shortgrass steppe ecosystem is dominated by blue grama (C_4) (Lauenroth

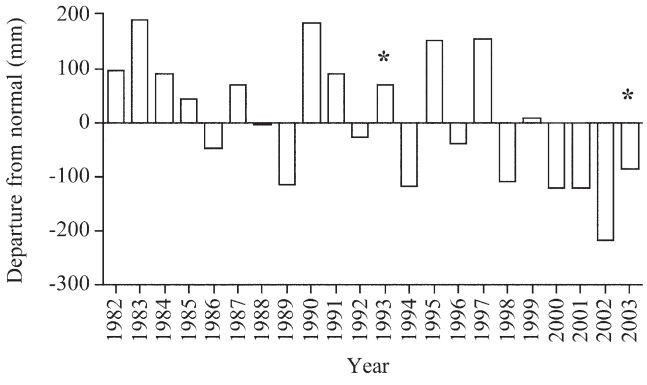

Fig. 2. Departure from the long-term (1977–2005) mean annual precipitation (428 mm) at the High Plains Grassland Research Station (HPGRS), Cheyenne, WY. The study was initiated in 1982 and sampled in 1993 and 2003 (indicated by the asterisks).

and Milchunas, 1979), which further substantiates the findings that soils supporting shallower rooted plant species would be prone to greater C loss because of the near-surface deposition/accumulation of organic C, via roots, relative to the deeper pattern of root growth in C_3 grass communities.

As plants become dormant in late summer/autumn, or during periods of low rainfall, rangeland soils can become net sources of CO_2 (Dugas et al., 1999; Sukyer and Verma, 2001; Frank, 2004). Research on South African semiarid, savanna ecosystems has noted that short bursts of rainfall can lead to "spikes" of soil microbial respiration (Veenendaal et al., 2004). This flush of CO_2 production can continue until soil water content is high enough for foliage to start greening up (in some cases up to 7 mo) (Sukyer and Verma, 2001; Veenendaal et al., 2004). While much of the rainfall at the HPGRS occurs as small events (61% of events are ≤5 mm; data not shown) there were few differences in the size of rainfall events between 1982 and 1993 and 1994 to 2003, suggesting the overall reduction in rainfall amount and frequency drove changes in SOC.

Soil Total Nitrogen

Patterns of TN paralleled those of SOC both in terms of concentration and mass in 2003 (Table 1). While the concentration and mass of TN for the depth increments (0–5, 5–15, 15–30, and 30–60 cm) were always in the order of CL > EX > CH, significant differences were only present at the 0- to 5-cm depth increment. There were, however, significant differences in the mass of TN among the three treatments for each soil interval (0–15, 0–30, and 0–60 cm) (Table 1). Soil TN did not reflect the changes observed in SOC between 1993 and 2003 (Table 2) and there were no significant differences between 1993 and 2003 for any of the treatments. Though not significant, an increase in TN in the EX and CL treatments and a loss of TN in the CH treatment over the 10-yr period in the 0- to 60-cm depth increment, resulted in significant difference between the EX and CL treatments relative to the CH treatment in 2003 (Table 1). In comparison, there was no significant difference in TN among treatments in the 0- to 60-cm depth increment in 1993 (Table 2). The change in soil TN over this 10-yr period, combined with a reduction in SOC in the CH grazing treatment, led to a nonsignificant decrease in the C to N ratio of all treatments (Table 2).

The loss of TN from the 0- to 60-cm soil interval (Table 2) under the CH, and increase in TN in the CL treatment, that occurred over the 10-yr study period is difficult to explain though numerous possibilities exist. Though increased forage consumption in the CH treatment resulted in a loss of approximately 50% (compared to a 10% loss in the CL treatment) of aboveground plant N (Schuman et al., 1999), it is estimated that 80 to 95% of consumed plant N is returned in the form of excreta (Heady and Child, 1994). A large proportion of the labile forms of N present in livestock excreta is unable to be taken up by plants and end up being either volatilized or denitrified (Whitehead, 1995), though it is likely this represents only a small component of overall N loss (Luo et al., 1999). In addition, it is possible that native legumes (listed within the 'Perennial forbs' classification, Table 3) present in the CH treatment have declined due to increased utilization but conversely had increased in the EX and CL treatments. Finally, we cannot

rule out that a proportion of the N perceived to be lost was, in fact, simply redistributed from the sampling areas to areas closer to water sources where animals congregate (Heady and Child, 1994).

Nitrogen Mineralization, Microbial Communities, and Activity

Potential N mineralization in the laboratory paralleled SOC and TN with CL > EX > CH (Table 4). Results of the N-mineralization field study, while not significant, showed a similar response as the laboratory study with CL having a weighted (by incubation period) mean value over the course of the growing season of 0.27 followed by CH at 0.22 with the EX treatment having the slowest rate of 0.21 μg N_i g^{-1} soil d^{-1}. In concert with field N-mineralization rates, we also calculated plant uptake of N_i. There were no significant differences in weighted means between treatments (EX = 0.16, CL = 0.22, and CH = 0.09 μg N_i d^{-1}). We hypothesize multiple factors were responsible for the nonsignificant differences in N mineralization among the treatments that were observed in both the field and laboratory N-mineralization experiments. They include greater productivity of the C_3 plant communities present in the EX and CL treatments and a subsequent increase in litter production (Table 3). It is likely that this litter would have a lower C to N ratio, relative to litter production from C_4 plants (Wedin and Tilman, 1990), leading to a less recalcitrant substrate (Ganjegunte et al., 2005) with higher rates of decomposition and turnover leading to greater substrate availability (Booth et al., 2005). In addition, greater soil moisture over the course of the growing season (EX, 5.8; CL, 6.2; CH, 4.3% mean soil moisture) would also have contributed to greater rates of field N mineralization in the EX and CL treatments. Other studies on northern mixed-grass prairies have also observed that as grazing intensity increased so did rates of root decomposition and N release, peaking under moderate gazing and declining under heavy grazing (Biondini et al., 1998).

Numerous field studies have examined the effect of grazing on N mineralization with mixed results. Increases (Frank et al., 2000; Johnson and Matchett, 2001), decreases (Ruess and McNaughton, 1987) and variable responses (Verchot et al., 2002; Patra et al., 2005) of N-mineralization to grazing have all been reported. Relatively few studies have specifically examined the affect of cattle (*Bos taurus*) grazing in mixed-grass, prairie ecosystems (Shariff et al., 1994; Biondini and Manske, 1996; Biondini et al., 1998, Wienhold et al., 2001). It is difficult to directly compare results among studies due to differences in grazing treatments and methodologies for measuring N mineralization. On average though, moderate levels of grazing have been found to result in higher rates of N mineralization relative to ungrazed or heavily grazed areas (Shariff et al., 1994; Biondini and Manske, 1996; Biondini et al., 1998). In this study, we can only speculate as to whether the decrease in N mineralization measured in the CH treatment was the result of SOC and/or TN losses, or because increases in C_4 plant production led to increase in the C to N ratio of above- and belowground litter and a subsequent decline in N mineralization (Wedin and Tilman, 1990). Schuman et al. (1999), however, reported few appreciable differences in above- or belowground C to N ratios of plant litter. Results of the laboratory study show the amount of potentially mineralizable N as a proportion of TN were 1.13, 0.98, and 0.84% in EX, CL, and CH treatments respectively, suggesting substrate quality (N and/or C) became progressively lower with an increase in grazing pressure (Ganjegunte et al., 2005). Regardless of the reasons behind the lower rates of N mineralization, a decrease in N availability will have profound and long lasting impacts in this low fertility, prairie ecosystem.

Table 4. Potential N mineralization (Nmin), potential microbial respiration (MR), and microbial biomass (MB) of soils from exclosed (EX), continuously, lightly grazed (CL), and continuously, heavily grazed (CH) pastures sampled in 2003 at the High Plains Grasslands Research Station (HPGRS), Cheyenne, WY.

Soil depth	Treatment	Nmin	MB	MR
cm		μg N_i g^{-1} soil	mg C g^{-1} soil	
0–5	EX	24.2[a]†	0.42[ab]	0.62[a]
	CL	25.5[a]	0.48[a]	0.68[a]
	CH	14.9[a]	0.29[b]	0.42[b]
5–15	EX	11.9[a]	0.13[a]	0.20[a]
	CL	12.2[a]	0.12 [a]	0.26[a]
	CH	8.8[b]	0.10 [a]	0.26[a]

† For a given parameter and depth, letters that are different from one another indicate a significant ($P \leq 0.10$) grazing treatment response.

Measures of MB (as determined by CFI) and M_R generally followed a pattern of CL > EX > CH (Table 4). The CH treatment was significantly lower in the 0- to 5-cm soil depth for MB than the CL treatment. Microbial respiration was significantly higher in the CL and EX treatments than the CH treatment for the 0- to 5-cm depth (Table 4). Microbial PLFA biomarkers were fairly consistent in the 0- to 5- and 5- to 15-cm depth increments with CL > EX > CH though there were no significant differences between any of the microbial groupings based on PLFA biomarker concentrations (Table 5). There were no diversity differences among treatments (Table

Table 5. Phospholipid fatty acid analysis of microbial groups in soils from exclosed (EX), continuously, lightly grazed (CL), and continuously, heavily grazed (CH) pastures at the High Plains Grasslands Research Station (HPGRS), Cheyenne, WY in 2003.

		Grazing treatment		
Soil depth	Microbial group	EX	CL	CH
cm		–nmol g^{-1} soil–		
0–5	Gram positive bacteria	15.0†	15.4	14.4
	Gram negative bacteria	9.8	10.9	8.2
	Actinomycetes	9.0	9.5	9.1
	Fungi	2.9	3.2	2.6
	AMF	2.3	2.5	1.9
	Protozoa	0.5	0.3	0.3
	Eubacteria	5.0	5.3	4.7
	Bacteria:Fungi‡	1.8:1	1.6:1	1.9:1
	Shannon diversity index	2.8	2.8	2.8
5–15	Gram positive bacteria	7.6	8.6	8.3
	Gram negative bacteria	4.1	5.4	4.1
	Actinomycetes	5.3	6.1	5.6
	Fungi	0.6	0.7	0.8
	AMF	1.0	1.2	1.0
	Protozoa	0.0	0.0	0.0
	Eubacteria	2.4	3.0	2.5
	Bacteria to fungi ratio	3.9:1	4.2:1	3.2:1
	Shannon diversity index	2.7	2.7	2.7

† For a given depth, there were no significant differences among treatments for any microbial group at $P \leq 0.10$.

‡ The bacteria to fungi ratio was calculated as the ratio of the eubacterial and fungal biomarkers.

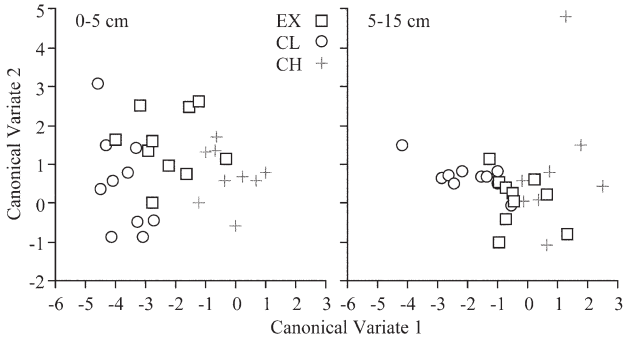

Fig. 3. Canonical variate analysis for microbial groups as determined by phospholipids fatty acid (PLFA) analysis for the 0- to 5- and 5- to 15-cm depth increments from soils in exclosed (EX), continuously, lightly grazed (CL), and continuously, heavily grazed (CH) pastures at the High Plains Grasslands Research Station (HPGRS), Cheyenne, WY.

5). For both depths, only canonical variate 1 was significant, explaining 89% of the variance observed in the 0- to 5-cm depth analysis and 92% of the variance in the 5- to 15-cm depth analysis (Fig. 3). Correlation analyses between canonical variate 1 and the sum of all the biomarkers (Pearson correlation coefficient = –0.36, P = 0.05) indicated that the separation that occurred at the 0- to 5-cm depth was, in part, the result of different amounts of biomass present in each of the three treatments. In contrast, at the 5- to 15-cm depth there was no significant correlation between the canonical variate 1 and the sum of the biomarkers (Pearson correlation coefficient = –0.22, P = 0.23) indicating that the separation observed among the three treatments was a result of differences in microbial community structure. In the 0- to 5-cm depth analysis, gram negative bacteria had a large negative loading on canonical variate 1 while actinomycetes, protozoans, and mycorrhiza had the largest positive loadings on canonical variate 1. In the 5- to 15-cm depth gram negative bacteria again had a large negative loading on canonical variate 1, while gram positive bacteria, actinomycetes, fungi, protozoa, and mycorrhiza all had positive loadings. This suggests that at both depths gram negative bacteria were found in greater quantities in the CL treatment compared to the CH treatment.

Greater MB (CFI and PLFA; Tables 4 and 5) and M_R (Table 4) in the CL and EX relative to the CH treatment were probably the result of the same factors as for N mineralization (i.e., greater amounts of high quality substrate and higher soil moisture). This hypothesis was further supported by strong correlations between SOC and MB (r^2 = 0.91, P = 0.0001; n = 12) and SOC and M_R (r^2 = 0.85, P = 0.0001; n = 10). Differences in root exudates among treatments may play an important role in affecting MB and M_R (Bardgett et al., 1998; Hamilton and Frank, 2001). Western wheatgrass roots have total nonstructural carbohydrate (TNC) concentrations that range from approximately 90 to 130 mg g^{-1} of root whereas TNC in blue grama roots are typically <55 mg g^{-1} of root (Buwai and Trlica, 1977). Consequently, there would have been a large source of organic C present in the root biomass of the EX and CL treatments as compared to the CH treatment. This would potentially give rise to a large source of labile C available for utilization by microbes (Dormaar, 1975). It would appear that in these semiarid ecosystems, labile forms of C and to a lesser degree, N, might be limiting many processes that are critical for nutrient cycling. This in turn may be part of a negative feedback loop in which overall nutrient cycling is 'slowed down' making it more likely that C_4 plants—with their more efficient use of N (Wedin and Tilman, 1990; Long, 1999)—will increasingly continue to dominate these systems.

The influence of grazing on MB is highly variable and seems to depend, to a large extent, on the amount of forage used, with increased grazing intensities leading to a decline in MB compared to ungrazed or more lightly grazed areas (Logakanthi et al., 2000; Li et al., 2005, Clegg, 2006). In contrast to our CFI estimates of MB (Table 4), we found no differences among treatments in total PLFA concentration or in the concentration of specific biomarkers (Table 5). Other grazing studies in which PLFA analyses have been undertaken have also found few differences between grazing or simulated grazing treatments (Patra et al., 2005; Zhang et al., 2005; Clegg, 2006), though Bardgett et al. (1997) noted a decrease in the PLFA fungal biomarker, 18:2ω6, with the removal of sheep (*Ovis aries*) grazing. Across three different submontane biogeographical areas, Bardgett et al. (2001) noted a general increase in total PLFA concentration with increased grazing pressure followed by a decline as sites became more heavily grazed. In all cases, though, lightly grazed sites contained maximal amounts of PLFA. Although differences in concentration of biomarkers of specific microbial groups were not significant among treatments (Table 5), canonical variate analysis indicated that the structure of soil microbial communities in the three treatments were statistically different from one another (Fig. 3). If we assume CL is the treatment that most closely resembled precattle grazing on these prairies (these prairies evolved over many millennia with generally light ungulate grazing), it appears soil microbial communities in the EX and CH treatments have shifted away from the CL treatment in terms of biomass at the 0- to 5-cm depth and structure at the 5- to 15-cm depth (Fig. 3).

Overall, our results generally contradicted the hypothesis put forward by Bardgett et al. (1998) that "Heavy grazing and hence frequent or more severe defoliation favors 'fast cycles' dominated by labile substrates and bacteria, while light grazing supports 'slow cycles' dominated by more resistant substrates and fungi." In our current study we found C and N mineralization to be 'faster' under the CL relative to the CH treatment (Table 4) suggesting that substrates were more labile. In addition we found that the CL treatment had greater concentrations of both fungi and bacteria as well as a lower bacteria to fungal ratio, compared to the CH treatment (Table 5). Light to moderate rates of grazing appears to give rise to not only the maintenance of soil physical and chemical properties, but also maximize the availability of resources for the soil biota and the many processes they control.

CONCLUSIONS

Many studies have noted that grazing typically results in small and usually nonsignificant changes to ecosystem functioning (provided overgrazing is not occurring), and that climate plays a much greater role in ecosystem processes. Grazing of mixed-grass prairie at rates not typically considered to be overgrazing (in this case a moderate rate of grazing using 50% of NPP), changed the vegetation composition from a C_3 domi-

nated, to a more C_4 dominated plant community. This change in plant community can lead to SOC accumulating closer to the soil surface, making it more vulnerable to being lost to the atmosphere, via CO_2 efflux, during periods of low rainfall/drought. Loss of C to the atmosphere is further exacerbated by changes in climatic conditions, specifically reduced precipitation and increased soil temperature. With the loss of SOC (as a proxy for SOM) there is also a decline in nutrients that are intimately associated with SOM (i.e., N) and this, in turn, may negatively impact microbial biomass and the processes that soil microorganisms control. The observed changes in C and nutrient cycling as a result of grazing may have long-term ramifications for sustainable production on grazing lands as well as for global climate change predictions.

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