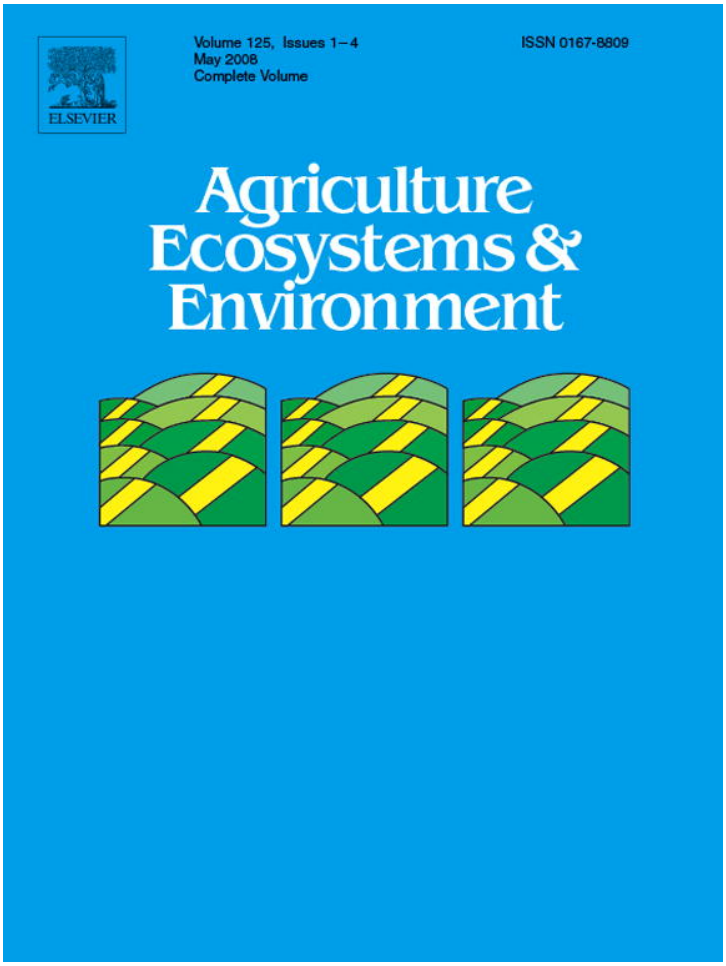

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Carbon accumulation and storage in semi-arid sagebrush steppe: Effects of long-term grazing exclusion

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Abstract

The potential of grazing lands to sequester carbon has been investigated in different terrestrial environments but the results are often inconclusive. Our study examined the soil organic carbon (SOC) and soil microbial biomass carbon (MBC) contents inside and outside four grazing exclosures that had been established more than four decades ago in the semi-arid sagebrush steppe of Wyoming. Non-grazed soil carbon parameters were compared to those of the adjacent grazed soils to examine the effects of long-term grazing exclusion on the soil carbon accumulation and storage of this particular ecological region. Soil organic carbon concentration in these soils ranged from 3.67 to 53.8 mg g^{-1} dry soil. There was no significant difference in SOC due to treatment (grazing exclusion) in three of the four sites. Carbon to nitrogen ratios ranged from 10 to 11 with only one site exhibiting greater C:N ratio in ungrazed soil than in grazed soil. Microbial biomass carbon concentrations ranged from 99 to 1011 μg g^{-1} dry soil in the study sites. All pairwise comparisons (with correlation coefficients from 0 to 1 at $\alpha = 0.05$ level) between MBC and SOC were significantly positive and strong for ungrazed soil in all four sites. Greater MBC was observed in the ungrazed soil than in the grazed soil at two sites, demonstrating that long-term grazing exclusion promoted enrichment of the labile soil carbon pool.

Keywords: Soil microbial biomass; Soil organic carbon; Grazing exclusion; Sagebrush grassland; Carbon sequestration; Land management

1. Introduction

Soils that have lost organic carbon through degradation may potentially act as efficient sinks of carbon under the appropriate natural (e.g. climate) and anthropogenic (e.g. land management) conditions (Batjes, 1999). Grazing lands may have a high carbon sequestration potential if the input of organic matter into soil and reduction of soil organic matter decomposition is promoted through best management practices (Batjes, 1999) because soil in many of them receive low carbon input and tend to be degraded, poorly managed or not managed at all (Kimble et al., 2001).

Studies of grazed soils worldwide have shown both increases (Schuman et al., 1999; Reeder et al., 2004) and decreases (Derner et al., 1997; Yong-Zhong et al., 2005) in carbon storage and accumulation compared to adjacent ungrazed soils. Milchunas and Lauenroth (1993) conducted a review of 34 studies involving grazed and ungrazed sites around the world and found that 40% of them reported a decrease and 60% reported an increase in soil carbon as result of grazing exclusion. According to Schuman et al. (1999), grazing can stimulate C and N cycling from aboveground plant components to the soil. They pointed out that animal traffic during grazing might enhance physical breakdown of organic matter, its incorporation into the soil, and rate of litter decomposition. Compared to areas grazed for 12 years after over 40 years of grazing exclusion, they found lower total C and N in non-grazed exclosure soils. Yong-Zhong et al. (2005) reported that soil organic carbon (SOC) and total nitrogen at a depth of 0–15 cm increased

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after 10 years of grazing exclusion in degraded sandy grasslands of northern China. From the study of C:N ratios, they observed that impacts of continuous grazing and grazing exclusion were more prominent on organic carbon than total nitrogen. Derner et al. (1997) found SOC and N at 0–5 cm was lower in the long-term (>25 years) grazed soils than in long-term ungrazed soils in North American Great Plains.

The soil carbon pool, microbial biomass and soil structure are interlinked during carbon accumulation and storage by the soil (Sparling, 1992; Breland and Eltun, 1999; Fließbach and Mäder, 2000; Nilsson, 2004). Sparling (1992) found that microbial biomass carbon (MBC) and the ratio of MBC and soil SOC can provide a more sensitive measurement of SOM than SOC data alone under forest soils for New Zealand topsoils. He found that MBC comprised 1–4% of total SOC with higher proportions under pastures than under forest soils. These data are related to Jenkinson and Ladd (1981) who mentioned that MBC comprise 1–3% of total SOM.

Carbon accumulation patterns in soil have also been found to differ according to depth and the vegetation it supports. Jobbágy and Jackson (2000) observed that the percentage of SOC averaged 33% and 42% in the top 20 cm of soil, relative to the first meter, for shrublands and grasslands, respectively. This was attributed to the differences in root distributions and above- and belowground biomass allocation patterns. The upper 30–40 cm of soil has approximately 80% of root biomass and with the highest concentration of SOM. This range of depth has hence been the depth most sampled during studies of SOM dynamics in grazing lands (Povirk et al., 2001). However, a study conducted by Derner et al. (1997) showed that maximum SOC and N accumulation was restricted vertically to the 0–5 cm depth and horizontally within the basal area under caespitose grasses. Our study, in agreement with prior studies, incorporated the analysis of both SOC and MBC in soils from two depths and three types of microsites: shrubs, grasses and bare interspaces.

Dominated by sagebrush (*Artemisia tridentata*) and wheat grasses (*Agropyron* spp.), the sagebrush steppe of Wyoming is exposed to severe winds, grazing, burning and variations in precipitation and temperature (World Wildlife Fund, 2001). Between years 1959 and 1965, with the objective of studying the effect of grazing on sagebrush steppe vegetation, approximately 100 exclosures were established in central Wyoming as part of a cooperative agreement between the University of Wyoming's Range Management Section and the Bureau of Land Management. Between years 2003 and 2005, with the objective of studying the effects of long-term grazing exclusion on the soil of this ecosystem, we analyzed SOC and soil MBC in four of those grazing exclosures.

2. Methods

2.1. The study sites

Study sites (Table 1) were located in the semi-arid sagebrush steppe region of Fremont County, Wyoming, USA (Fig. 1). The vegetation type in all exclosures is sagebrush steppe grassland (Monger and Martinez-Rios, 2001) with Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *Wyomingensis* Beettle and Young) as the most dominant shrub. Soil textures at the study sites ranged from sandy loam to clay loam (Table 2). Average annual precipitation between years 1990 and 2003, for all four sites approximated 200–210 mm while the mean elevation ranges from 1600 to 2100 m. Soil at GM and UGD exclosures belongs to the mixed frigid Ustic Haplargid family while soil at Shon 8 and Shon 9 exclosures belongs to the mesic Ustic Haplargid family (USDA-NRCS, 2005). All four sites had Haplic Yermosols, based on FAO soil classification.

Table 1
Description of study sites in Wyoming[a] with US soil classification[b]

Site[c]	County	Soil type	Elevation (m)	Average precipitation[d] (mm)	Establishment (year)	Area (m^2)	Stocking rate[e] (AUM ha^{-1})
Granite Mountain (GM)	Fremont		2166.5	213	1962	61 × 121	0.3[f]
Upper Government Draw (UGD)	Fremont	Mixed frigid Ustic Haplargid (Haplic Yermosols)	1853.2	200	1958	107 × 203	0.2[g]
Shoshoni Ant # 8 (Shon 8)	Fremont		1597.1	203	1964	183 × 183	0.03[h]
Shoshoni Ant # 9 (Shon 9)	Fremont	Mesic Ustic Haplargid (Haplic Yermosols)	1591.1	203	1964	183 × 183	0.03[h]

[a] Data were collected from the Bureau of Land Management, Lander, WY archives in June 2004.
[b] Soil classification based on FAO are listed within brackets.
[c] Abbreviations are given within brackets.
[d] Average annual precipitation are for the years between 1960 and 2003.
[e] Approximate stocking rates in allotments or corresponding pasture within that allotment; data from 1993 to 2003; compiled from the Bureau of Land Management, Lander, WY archives.
[f] Rotational grazing by livestock from May to November (190 days). Sheep grazing in May, June and September.
[g] Two pastures deferred rotational grazing by livestock from May to July (76 days).
[h] Three pastures deferred rotational grazing from December to April (44 days) by cattle and sheep.

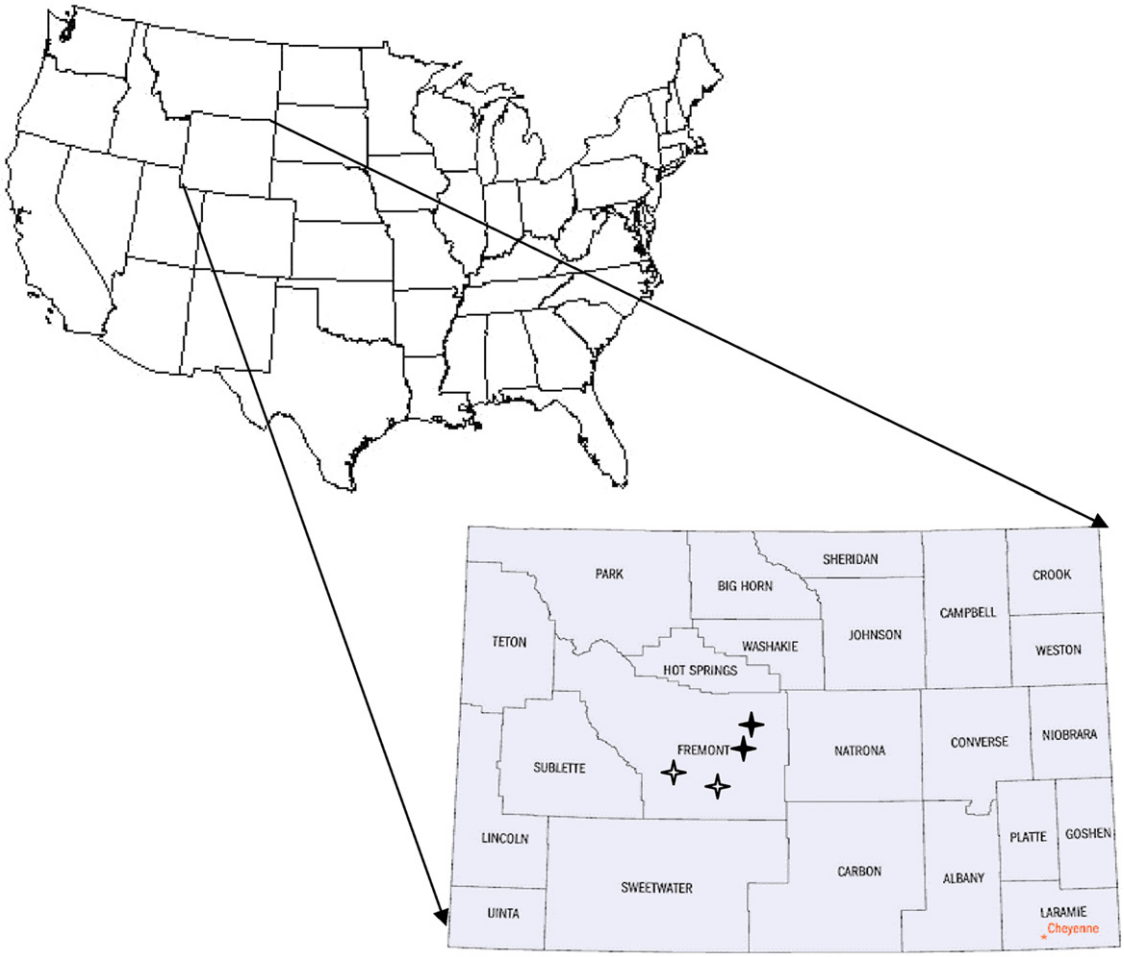

Fig. 1. Study sites in central Wyoming, coterminous US. White stars denote GM and UGD sites; solid black stars denote Shon 8 and Shon 9 sites.

Table 2
Dominant vegetation and soil characteristics of the study sites

Site	Grasses[a]	Forbs[a]	Shrubs[a]	Shrub cover[a] (%)	Sand:silt: clay[3] (%)	Soil texture[b]	pH[b]	EC[b] (μS/cm)
GM	*Agropyron smithii*, *Poa secunda*, *Agropyron spicatum*, *Oryzopsis hymenoides*	*Phlox hoodii*, *Eriogonum sphaerocephalum*, *Crepis acuminate*, *Antennaria dimorpha*	*Artemesia tridentata*, *Gutierrezia sarothrae*, *Chrysothamnus viscidiflorus*	19 (in) 28 (out)	56:29:15	Sandy loam/loam	6.8	137.7
UGD	*Agropyron smithii*, *Poa secunda*, *Agropyron repens*, *Koeleria cristata*	*Crepis acuminate*, *Phlox hoodii*, *Opuntia polyacantha*, *Alnus tenuifolia*, *Eriogonum sphaerocephalum*, *Sphaeralcea coccinea*, *Carex* spp.	*Artemesia tridentata*, *Gutierrezia sarothrae*	29 (in) 26 (out)	40:40:20	Loam/clay loam/silt loam	6.0	103.3
Shon 8	*Agropyron smithii*, *Poa spp.*, *Kochia Americana*, *Stipa Columbiana*,	*Alnus tenuifolia*, *Eriogonum sphaerocephalum*, *Ludwigia sphaerocarpa Opuntia polyacantha*	*Artemesia tridentata*	18 (in) 28 (out)	67:18:14	Sandy loam	6.7	88.4
Shon 9	*Bouteloua gracilis*	*Alnus tenuifolia*, *Eriogonum sphaerocephalum*, *Ludwigia sphaerocarpa Opuntia polyacantha*, *Carex spp.*, *Lobelia spicata*	*Artemesia tridentata*	24 (in) 20 (out)	67:20:13	Sandy loam	6.7	95.1

[a] *Source*. UWYO Exclosure Database (2003). 'In' refers to inside the exclosure (ungrazed site) and 'out' refers to outside the exclosure (grazed site).
[b] Texture, pH and EC analyses were done during this study. Mean values are given here.

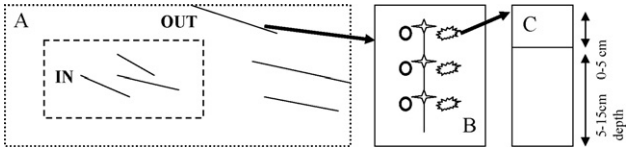

Fig. 2. Sampling design for the study. (A) '– –' denotes the fence of the exclosure; '. . .' is an imaginary boundary outside the exclosure. Transects are denoted by straight unbroken lines. (B) Transect showing microsites grasses, shrubs and bare interspaces. (C) Two sampling depths under each microsite.

Studied exclosures had minimal external disturbance and fence breaches since time of establishment (years 1958–1964). Average stocking rates (years 1993–2003) outside the four exclosures were approximately 0.3, 0.2, 0.03 and 0.03 AUM/ha for GM, UGD, Shon 8 and Shon 9 exclosures, respectively (BLM District Office, Lander, WY).

2.2. Experimental design and soil sampling

The basic experimental design (Fig. 2) was a one-factor experiment with three levels (treatment, microsite, depth). Soil sampling was conducted separately both inside and outside the grazing exclosures, along three 100 m long transects spaced at least 20 m apart. Transects were laid entirely inside and outside the exclosures, with three sampling points along each. The area inside an exclosure was the 'ungrazed' treatment while the land outside was the 'grazed' treatment. At each sampling point, samples were collected from under three microsites: sagebrush, grasses and bare interspaces—and at two soil depths (0–5 cm and 5–15 cm). The total number of samples per treatment in each site was 54 ($3 \times 3 \times 3 \times 2$). Each sample was sealed in ziplock bags and put in coolers. They were transported to the soil storage facility at the University of Wyoming, air-dried in the greenhouse and stored under refrigerated conditions (4 °C).

2.3. Sample preparation

All soil samples were air-dried, dry-sieved through a 2 mm sieve to remove rocks and plant materials and stored airtight in refrigerated space. At each phase of subsequent analyses, visible plant materials and other non-soil materials were removed from the samples by means of forceps. The air-drying process that we employed has very low to no possibility of altering the natural soil MBC conditions as the samples were collected from a dry region during the summer when microbial populations were naturally exposed to temperatures above 32 °C for several weeks. To further minimize any possible effects of sampling time, soil temperature and moisture status of the soils during sampling time (Broos et al., 2007), the air-dried soil samples were brought to 50% water holding capacity and incubated in the dark at room temperature for 10 days before MBC analysis. Before total C and N analyses, bulk soil was finely ground and homogenized by using the roller-milling device as described by Arnold and Schepers (2004) for carbon and nitrogen analyses. Prior to grinding, 5–6 g soil from each sample was transferred into square glass bottles with three stainless steel rods approximately 4.7 cm long and 0.45 cm diameter. Bottles were capped, aligned on the roller miller and ground by the combined effect of the rotating bottles containing soil samples and steel rods on the miller for 24 h. Ground soils were placed back into their original ziplock bags and stored at room temperature until further analyses.

2.4. Soil carbon analysis

The dry combustion method was employed for determination of total carbon. A Carlo Erba 1500 CN Analyzer (Carlo Erba, Milan, Italy) was used to measure total C and N concentrations for the 216 samples from the GM and UGD exclosures. The Elementar Variomax CN Analyzer (CN analyzer, Elementar Americas, Inc., Mt. Laurel, NJ) was used for the 216 samples from Shon 8 and Shon 9. From each soil sample, 300 mg subsamples were taken for these analyses. Total N, total C and C:N ratios were obtained. The modified pressure calcimeter method, as described by Sherrod et al. (2002), was used for analysis of inorganic carbon (IC) in fine-ground soil samples. Soil organic carbon content was calculated by subtracting the inorganic carbon content from the total carbon content of each soil sample. To calculate organic carbon concentration per hectare of land in each study site, mean SOC concentrations, soil bulk density (BD) (from samples collected with hammer driven bulk density core by Madden, 2005) and soil depths for each soil sample were plugged into the following formula:

$$\mathrm{SOC}\ (\mathrm{Mg\,ha^{-1}}) = \mathrm{BD}\ (\mathrm{g\,cm^{-3}}) \times \mathrm{soil\ depth}\ (\mathrm{cm}) \times \mathrm{mean\ SOC}\ (\mathrm{mg\,g^{-1}}) \times 10^{5} \quad (1)$$

2.5. Soil microbial biomass analysis

The chloroform fumigation–extraction method described by Vance et al. (1987) and Howarth and Paul (1994) was used for quantifying MBC. Organic (microbial) carbon concentration in each sample (control and chloroform-fumigated) was determined using a Phoenix 8000 UV-Persulphate Total Organic Carbon Analyzer (Teldyne-Tekmar, Mason, Ohio). The difference between the carbon in the controls and chloroform-fumigated samples was utilized to calculate the MBC with the following equation:

$$\mathrm{MBC} = \frac{C_{\mathrm{fumigate}} - C_{\mathrm{control}}}{K_{\mathrm{ec}}} \quad (2)$$

where K_{ec} is the correction factor related to the proportion of microbial biomass or the coefficiency of extracting microbial carbon from the soil; C_{control} the microbial biomass carbon from the control (unfumigated) samples and C_{fumigate} is the microbial biomass carbon from the fumigated samples. A K_{ec} value of 0.37 was used for our calculations.

2.6. Statistical analyses

Statistical analyses, except correlation matrices, were conducted using Minitab 13.1 (MINITAB 2000). General linear model (GLM) was employed for analysis of variance (ANOVA) between treatments, microsite and depths within a site. Tuckey's post hoc analysis was used for significant interactions and for significant factor comparisons. A confidence interval of 95% ($\alpha = 0.05$ level of significance) was used for analysis of significant difference unless stated otherwise. Dependent variables analyzed were SOC and MBC. Independent variables were treatment, microsite and depth. For analysis of overall means across all microsites and depths within treatments, two-sample *t*-test was employed. As mentioned earlier, the total number of samples from each site was 108 (i.e. 54 from each treatment, two depths and three microsites) for all analyses. Fishers' *r*–*z*-transformation and significance test at $\alpha = 0.01$ was employed for correlation matrices of SOC, MBC and C:N ratios using STATVIEW 5.0.1 program (SAS Institute, 1992–1998). Presentation of statistical results has been done with an emphasis on the effect of treatment, as a main effect or as part of a significant interaction. The main objective of the statistical analyses was to examine differences due to treatment (grazed vs. ungrazed) on the studied parameters. Interactions involving treatment with depth and/or microsites were also analyzed and reported when significant.

3. Results

3.1. Soil organic carbon concentration

SOC concentration in the studied soils ranged from 3.7 mg g^{-1} dry soil (0.37%) in Shon 8 grazed bare interspace soil (5–15 cm) to 53.8 mg g^{-1} dry soil (5.4%) in GM ungrazed soil (0–5 cm) under shrubs. Statistical analyses of variance using GLM revealed that only the GM site demonstrated significant differences in SOC due to treatment although significant interactions involving treatment, microsite and/or depth were observed for all the sites (Table 3). Two-sample *t*-test of the overall SOC means incorporating all depths and microsites showed the absence of significant difference between grazed and non-grazed soils at all the sites (Table 4).

Table 4
Statistical comparison of overall mean values of SOC content, SOC ha^{-1} and C:N ratio (incorporating all depths and microsites) ± standard error (S.E.) using two-sample *t*-test ($\alpha = 0.05$) between grazed and ungrazed treatment within each site

	GM	UGD	Shon 8	Shon 9
SOC dry $soil^{-1}$ ± S.E. (mg g^{-1})				
Ungrazed	22.7 ± 0.21	11.4 ± 0.08	8.3 ± 0.09	6.8 ± 0.06
Grazed	19.4 ± 0.17	12.47 ± 0.13	7.2 ± 0.06	7.2 ± 0.08
P-value	0.654	0.756	0.297	0.618
SOC ha^{-1} ± S.E. (Mg ha^{-1})				
Ungrazed	9.2 ± 2.83	6.8 ± 2.61	5.8 ± 1.12	15.7 ± 6.47
Grazed	10.1 ± 2.43	6.3 ± 2.42	5.9 ± 1.77	15.2 ± 4.08
P-value	0.54	0.72	0.91	0.87
C:N ratio mean ± S.E.				
Ungrazed	11.2 ± 0.2	11.0 ± 0.19	10.8 ± 0.26	10.8 ± 0.20
Grazed	11.2 ± 0.2	10.9 ± 1.9	11.3 ± 0.27	9.9 ± 0.33
P-value	0.898	0.552	0.241	0.026

For SOC data obtained from the GM exclosure, ANOVA indicated a significant three-way interaction among treatment, microsite and soil depth. This site was also the only one showing significant difference in SOC due to (grazing exclusion) treatment, based on ANOVA. Tuckey's post hoc analysis of means showed that ungrazed soil under shrubs at 0–5 cm depth had greater SOC content than grazed soil under shrubs at 0–5 cm depth ($P < 0.001$) and soils from all other microsites and depths in both grazed and ungrazed treatments ($P < 0.001$). At both UGD and Shon 8 exclosure sites, a significant interaction of microsite and depth on SOC concentration was observed. No significant difference due to treatment was observed in both of these sites. Ungrazed soil at 0–5 cm depth from bare interspaces usually had lower SOC content than ungrazed soil under shrub canopies at the same depth. For the Shon 9 exclosure, there was no significant difference in SOC due to treatment. Grazing exclusion was seen to influence SOC in 0–5 cm soil more than in soil at 5–15 cm.

There were no significant differences due to treatment in calculated SOC content per hectare of land between grazed

Table 3
Partial ANOVA table showing degrees of freedom (DF) and *P*-values from GLM analysis (at $\alpha = 0.05$) of SOC in the study sites across treatments (ungrazed, grazed), microsites (bare, grass, shrub) and depths (0–5 cm, 5–15 cm)

Factors and interactions	DF	*P*-values			
		GM	UGD	Shon 8	Shon 9
Treatment	1	0.0362	0.1693	0.1121	0.4650
Microsite	2	<0.0001	<0.0001	<0.0001	<0.001
Depth	1	<0.0001	<0.0001	<0.0001	<0.0001
Treatment × microsite	2	0.0053	0.6843	0.3021	0.4760
Treatment × depth	1	0.0015	0.5716	0.6667	0.2222
Microsite × depth	2	<0.0001	<0.0001	<0.0001	<0.0001
Treatment × microsite × depth	2	0.0008	0.9597	0.8476	0.5561

Table 5
Partial ANOVA table showing degrees of freedom (DF) and *P*-values from GLM analysis (at $\alpha = 0.05$) of MBC in the study sites across treatments (ungrazed, grazed), microsites (bare, grass, shrub) and depths (0–5 cm, 5–15 cm)

Factors and interactions	DF	*P*-values			
		GM	UGD	Shon 8	Shon 9
Treatment	1	<0.001	0.0894	0.2182	0.002
Microsite	2	<0.0001	<0.0001	0.0062	<0.0001
Depth	1	<0.0001	<0.0001	<0.0001	<0.0001
Treatment × microsite	2	0.2164	0.4385	0.2007	0.6071
Treatment × depth	1	0.0430	0.3130	0.8328	0.2564
Microsite × depth	2	< 0.001	0.0922	0.1368	0.1888
Treatment × microsite × depth	2	0.3034	0.1844	0.0407	0.4117

and ungrazed soils in any of the sites (Table 4). At both GM and UGD exclosures, significant three-way interactions of treatment, microsite and depth on SOC ha^{-1} were observed, indicating effects of grazing exclusion on SOC ha^{-1} varied according to microsites and soil depths. At Shon 8 and Shon 9 exclosures, there were significant interactions of microsite and depth on SOC ha^{-1}. The soil bulk density values used in these calculations, as analyzed by Madden et al. (2005), ranged from 0.9 to 1.4 g cm^{-3}. No significant differences were reported for bulk densities between grazed and ungrazed soil within each of the four sites.

3.2. Carbon to nitrogen ratios

GLM analysis of soil C:N ratios showed no significant differences at any study sites except Shon 9 exclosure (Table 4) where greater C:N ratio was observed in the ungrazed soil than in the grazed soil. All C:N ratios ranged from 10 to 11.

3.3. Microbial biomass carbon

MBC concentrations ranged from 99 to 1011 μg g^{-1} dry soil in the study sites. The lowest MBC content was observed for grazed bare interspace soil at 5–15 cm in Shon 9. The highest MBC content was observed for ungrazed 0–5 cm soil under shrubs in GM.

Statistical analysis of MBC data using GLM (Table 5) showed significantly greater MBC in the ungrazed soil compared to grazed soil in two of the four study sites (GM with $P < 0.001$ and Shon 9 with $P = 0.002$), at the 95% level of confidence interval. In one site (UGD), MBC was greater in the ungrazed soil than in the grazed soil at the 90% level of confidence interval ($P = 0.0894$). At Shon 8 site, there was no significant difference in MBC between grazed and ungrazed soils. Greater MBC concentrations in ungrazed 0–5 cm depth soil under shrubs were generally observed compared to grazed soil from 5 to 15 cm depth in other microsites in all three study sites except Shon 8. Two-sample *t*-tests showed that the average of all MBC data within a site and treatment (inclusive of all microsites and depths) was

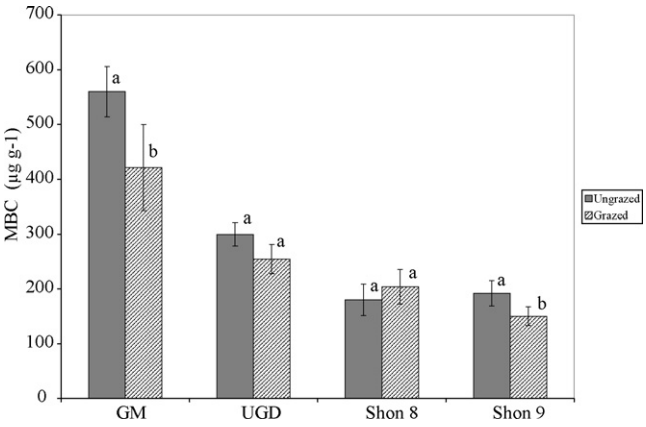

Fig. 3. Mean MBC concentration in grazed and ungrazed soils of all sites, inclusive of all microsites and depths. Error bars represent standard errors, $n = 54$ for each treatment within a site. Different letters between treatments within a site represent significant difference at 95% level of confidence interval.

significantly greater in ungrazed soil than grazed soil for both the GM site ($P = 0.0184$) and the Shon 9 site ($P = 0.0085$) (Fig. 3), similar to the two-sample to the ANOVA results above. This test showed no overall significant difference between treatments for the UGD site ($P = 0.1303$) and Shon 8 site ($P = 0.2885$).

3.4. Relationship among MBC, SOC and C:N ratio

The ratios of MBC:SOC were observed to be significantly greater in ungrazed soil than in grazed soil at the GM and UGD exclosures (Table 6). The mean MBC:SOC ratio was slightly, though not significantly, greater in the ungrazed soil than in the grazed soil at the Shon 9 exclosure. At Shon 8 exclosure, significantly greater MBC:SOC ratio was observed in the grazed soil than in the ungrazed soil.

All pairwise comparisons (with correlation coefficients from 0 at $\alpha = 0.05$ level) between MBC and SOC were significantly positive and strong for ungrazed soil in all four sites (Table 7). Granite Mountain was the only site where a significant correlation between MBC and SOC in the grazed soil was observed. Strong positive correlations were observed between MBC and C:N ratio in the ungrazed treatments at GM and Shon 9 exclosures. At the GM, UGD and Shon 9 sites, SOC and C:N ratio exhibited strong positive correlation for both grazed and ungrazed treatments. No significant

Table 6
Statistical comparison of average MBC:SOC ratios (incorporating all depths and microsites) using two-sample *t*-test ($\alpha = 0.05$) between grazed and ungrazed treatment within a site

Treatment	MBC:SOC ratio mean ± S.E. (mg MBC g^{-1} SOC)			
	GM	UGD	Shon 8	Shon 9
Ungrazed	26.8 ± 1.2	27.5 ± 2.0	25.8 ± 1.9	30.5 ± 1.8
Grazed	21.4 ± 1.6	22.2 ± 1.6	34.9 ± 2.6	28.6 ± 4.3
P-value	0.007	0.044	0.004	0.685

Table 7
Correlation matrices for SOC, MBC and C:N ratio in the study sites[a]

Site	Treatment	Parameter	SOC	C:N
GM	Ungrazed	MBC	0.723*	0.620*
		SOC		0.391*
	Grazed	MBC	0.521*	0.287
		SOC		0.565*
UGD	Ungrazed	MBC	0.379*	0.148
		SOC		0.752*
	Grazed	MBC	0.059	0.252
		SOC		0.756*
Shon 8	Ungrazed	MBC	0.455*	0.320
		SOC		0.230
	Grazed	MBC	0.120	0.001
		SOC		0.111
Shon 9	Ungrazed	MBC	0.641*	0.614*
		SOC		0.855*
	Grazed	MBC	0.302	0.240
		SOC		0.831*

* Significant difference of correlation coefficient from 0 at $\alpha = 0.05$ level ($P < 0.05$) following correlation Fishers' r–z-transformation and significance test.

[a] All values are Pearson correlation coefficient (range 0–1); $n = 42$ (ungrazed), $n = 52$ (grazed) for GM, $n = 33$ (ungrazed), $n = 35$ (grazed) for UGD, $n = 34$ (ungrazed), $n = 36$ (grazed) for Shon 8 and $n = 35$ (ungrazed), $n = 35$ (grazed) for Shon 9.

correlation between these parameters was observed at the Shon 8 site.

4. Discussion

4.1. Lack of significant change in SOC

Overall, our study showed lack of significant difference in SOC between grazed soil and soil not grazed for more than 40 years, in spite of significant interactions of treatment, microsite and/or depth. Kieft (1993) observed results similar to ours with lack of significant differences in SOC between grazed lands and land not grazed for 11 and 16 years. The low stocking rates in our sites could have reduced the loss of SOC in the grazed treatment. The average stocking rates in the study sites (Table 1) were low at GM and UGD while they were extremely low at Shon 8 and Shon 9. Reeder et al. (2004) observed a similar lack of significant difference in SOC between lightly grazed soil (20–35% utilization) and soil not grazed for 56 years in shortgrass steppe. Dormaar and Willms (1998) also observed no change in total soil carbon concentration after 42 years of light grazing (1.2 AUM ha^{-1}) 1) in Canadian fescue grasslands, although it was seen to decrease with increase in grazing pressure.

Different factors may have led to the absence of significant changes in SOC levels in the study sites. The average bulk densities of soil in the study sites also did not show significant difference between grazed or non-grazed treatments (Madden, 2005). There was no evidence of compaction due to grazing. The past and current low stocking rates may be exerting low influence on SOC levels. Our results may also differ from prior results showing grazing-induced SOC increase (Schuman et al., 1999; Reeder et al., 2004) or decrease (Derner et al., 1997; Yong-Zhong et al., 2005) because of differences in climate, soil and/or vegetation types. Other reasons may be (i) historical grazing practices and grazing intensities before and after grazing exclusion and (ii) grazing by small mammals inside the grazing exclosures (Kieft, 1993).

Our results are not consistent with a study in a mixed grass ecosystem by Schuman et al. (1999), who found greater SOC in grazed soil than in soil not grazed for 40 years. There are minor similarities with Hiernaux et al. (1999) who observed that after 4 years of controlled grazing in a Sahelian rangeland, SOC decreased slightly compared to ungrazed control but after 9 years of intensive grazing, SOC did not decrease further. Our results may have been different from these prior studies because our ungrazed sites had been grazed prior to exclusion and our grazed sites had been grazed for years. Hiernaux et al. (1999) conducted their grazing studies on previously ungrazed soils.

As mentioned earlier, recent grazing history in our study sites indicates light and well-managed grazing outside the four studied grazing exclosures. Well-managed grazing improves nutrient cycling in grassland ecosystems, stimulating aboveground production as well as root respiration and exudation rates (Schuman and Derner, 2004). As mentioned by Post and Kwon (2000), the length of time and the rate of carbon accumulation in the soil vary widely from one area to another, depending on the productivity of the vegetation recovering from environmental changes, physical and biological conditions in the soil and the history of soil organic carbon input and physical disturbance. Differences in methodology of sampling and analyses, plant response to grazing, and photosynthesis of grazed plants may be responsible for different results of grazing on SOC (Schuman et al., 2000).

4.2. Effect on carbon to nitrogen ratios

The C:N ratios were similar in three of the study sites regardless of grazing or non-grazing treatments. Shon 9 exclosure had slightly greater C:N ratio in the ungrazed soil. Dormaar and Willms (1998) observed similar results with very little change in C:N ratio due to light grazing for 42 years (C:N ratio of 13.4 in no grazing and 12.6 in light grazing treatment). In all the study sites, the C:N ratios were relatively low, indicating a low content of recently input and more labile SOM (Wander and Traina, 1996). Comprehensive studies and similar results of impacts of grazing exclusion on SOC and C:N ratios are not prominent in prior literature.

4.3. Changes in microbial biomass carbon

Although our study data indicated that long-term grazing removal did not significantly affect SOC, MBC did exhibit

significant changes. We observed greater MBC in the ungrazed soil than in the grazed soil in two of our four study sites. These results are similar to Fließbach and Mäder (2000) who observed early system-induced changes on soil MBC while comparing conventional and organic farming systems, whereas no such changes were observed on total SOM. Soil MBC was found to be more sensitive to impacts of grazing than SOC in Mongolian grasslands with greater microbial biomass carbon in ungrazed soil than in grazed soils (Ma et al., 2005). Ma et al. (2005) also observed that after 22 years of grazing, compared to a lower than 5% decline in SOC, the decline in soil microbial biomass carbon was almost 30%. Contrary to our results, Kieft (1993) did not observe significant and consistent changes on soil MBC due to grazing exclusion in grasslands of New Mexico.

4.4. The relationship of MBC with SOC

The MBC:SOC ratio was significantly greater in ungrazed soil compared to grazed soil at the GM and UGD sites. This indicates a greater abundance of readily metabolizable C, a more metabolically active microbial community and greater carbon turnover (Kieft, 1993) in the ungrazed site than in the grazed site. Shon 9 also had slightly greater, but not significantly different, means of MBC:SOC ratio in ungrazed soil than in grazed soil. In the ungrazed soil in these three sites, greater MBC:SOC ratios may have occurred due to greater amounts of organic matter accumulating in the soil and more suitable environment for microbial growth, through absence of physical disturbance, for microbial proliferation from available soil organic carbon. At Shon 8, significantly greater MBC:SOC ratio was observed outside the exclosure than within it.

Holt (1997) found lower MBC:SOC ratio in short-term heavily grazed soils, compared to lightly grazed soils. However, they also observed lack of difference in this ratio for long-term heavily grazed soils and lightly grazed soils, showing the decline of both MBC and SOC. Lack of other similar studies and results on effects of grazing exclusion on MBC and MBC:SOC ratios of the soil is evident.

The linear relationships from simple linear regression of MBC vs. SOC were positive for all four study sites. Strong positive relationships between SOC and MBC were observed in the ungrazed soil of GM, UGD and Shon 9 exclosures, compared to weaker relationships in the grazed soil. Correlations, though positively significant for both treatments, were stronger for the ungrazed treatment than for grazed treatment in those sites. Such a correlation was also observed in Shon 8 exclosure although it did not show a strong linear relationship between SOC and MBC in both treatments. Higher organic matter inputs from plant litter and root exudates in ungrazed soil, compared to lower organic input in the grazed soil, may have enhanced the rate of MBC production, a labile SOM faction, in the soil (Bird et al., 2002). Grazing may be acting as an external agent slightly disrupting this relationship outside the exclosures in those sites. Carbon to nitrogen ratios had a strong correlation with SOC for both grazed and ungrazed treatments in all three sites except Shon 8 exclosure, which showed weak correlation of C:N vs. SOC.

5. Conclusion

A study recently conducted by Raiesi and Asadi (2006) in semi-arid rangeland of central Iran bears close resemblance to ours. They observed no difference in SOC and C:N ratio between grazed soil and soil not grazed for 17 years in semi-arid range, also suggesting that that period may be insufficient for higher C accumulation in the soil or that grazing in these systems does not affect SOC levels. In ungrazed systems, more of the primary production goes directly into the soil, becoming substrates for microbes than in the grazed systems where herbivores consume a portion of the primary production. This difference in C cycling is apparently reflected more in MBC than in SOC. Data from our study indicate no significant overall changes in SOC after 40 years of grazing exclusion. Significant changes in MBC, however, indicate that grazing exclusion may have induced some differences in carbon dynamics and nutrient cycling in the soil. Grazing decreases soil respiration and microbial biomass C while overgrazing may reduce the input of fresh plant residue, living roots and exudates for stimulating microbial activity (Raiesi and Asadi, 2006). However, managed grazing outside the exclosures in our study sites appears to have stabilized the accumulation and storage of soil organic carbon.

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