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How does livestock graze management affect woodland soil health?

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Soil is the fundamental component of each terrestrial ecosystem, so the consequences of land management regime on soil health and productivity should be evaluated. To this end, the long term effects of livestock grazing management on soil health were studied in three land management systems of north-eastern Iran, comprising unlimited grazing, managed grazing, and a rangeland where grazing was prohibited. First, properties including pH, EC, bulk density and contents of phosphorus, potassium and calcium in soils subject to the three grazing management regimes were compared. Then, seasonal variations of organic carbon, total nitrogen, microbial respiration (BR and SIR), carbon and nitrogen of microbial biomass (MBC and MBN), fungal biomass and AMF (*Arbuscular mycorrhizal* fungi) spore density were studied at the three sites. Several soil stoichiometric microbial indicators were also compared. Soil phosphorus contents were found to drastically decline (by around 40%) in response to long term unlimited grazing, while soil acidity and bulk density slightly increased under that grazing regime, in comparison with managed-grazing and grazing-excluded rangelands. Season and grazing management had significant impacts on all the studied variables. Although soil nitrogen content increased, presumably due to livestock urine, organic carbon content and all the soil microbial variables declined in grazed sites, which were also the ones with lower plant cover. A higher AFM spore density was observed around the rhizosphere of *Artemisia aucheri*, the most frequent plant, in lands with unlimited grazing. Based on these results, managed grazing is strongly recommended rather than any kind of unlimited or continuous grazing.

KEYWORDS

livestock, soil properties, seasonal variations, microbial activity, plant cover

Introduction

It is estimated that rangeland ecosystems occupy about half of the world's land area (Schuman et al., 2002). The conservation, development and management of these valuable natural resources require knowledge and basic information. Most rangelands are threatened by both improper strategic management and over-grazing by livestock (Alemu, 2016). They have been exposed to such overgrazing for many years, which not only threatens their plant biodiversity and stability, but has also altered their ecosystem structure and function, leading to declines in soil productivity (Frank et al., 2000; Liu T. et al., 2011; Zhou et al., 2017). According to Tanentzap and Coomes (2012), proper grazing management can maintain and increase biomass productivity and subsequently improve soil organic matter.

Increasing soil organic matter in rangeland, where the plant community has not been extensively grazed by livestock, enriches the soil quality, microbial community, and microorganism biodiversity (Verdú et al., 2000; Dubeux et al., 2006; Zhang et al., 2018). On the other hand, any changes in the plant community composition due to overgrazing lead to shifts in soil ecophysiological functions (Barger et al., 2004; Pan et al., 2018). Moreover, deposition of dung and urine of livestock *via* grazing can substantially change soil fertility (Bolan et al., 2004), raising the question as to whether grazing improves or degrades the soil.

Although trampling by livestock compacts and modifies the physical structure of the soil (Silva et al., 2003), grazing also has less visible effects, notably on chemical and biological characteristics (Hashemi et al., 2019). Nutrient uptake (Farrakh Nawaz et al., 2013), soil microbial biomass (Pupin et al., 2009), soil enzymatic activities (Buck et al., 2000), and soil fauna (Chan and Barchia, 2007) can be negatively affected by soil compaction, but these shifts depend greatly on the characteristics (species, size, etc.) of the grazing flocks (Farrakh Nawaz et al., 2013).

Given the increasing human need for livestock products, grazing management is vital to maintain and support rangeland services. Along with common physical and chemical indicators for assessment of the sustainability of soil ecosystems, microbial activity is a reliable tool to study soil disturbance resulting from grazing (Piotrowska-Dlugosz and Charzynski, 2015). Understanding the relationships between grazing regime and soil microbial activities can provide useful information for sustainable use and proper management of rangeland (Qiu et al., 2013).

Most studies of the impacts of livestock grazing on soil ecosystems have focused on soil physical and chemical parameters (Binkley et al., 2003; Javadi et al., 2006; Piñeiro et al., 2010; Ajorlo et al., 2011; Karimian and Jafari, 2017) rather than soil microbial variables. For instance, Hashemi et al. (2019) utilized soil enzyme activities as soil biological indicators to assess the impact of livestock trampling on oak forest stands. Moreover, Kooch et al. (2020) employed some soil microbial activities such as microbial transpiration and microbial biomass carbon and nitrogen to determine the impact of grazing management on northern Iranian rangeland.

Soil microbial activity is a promising indicator of effects of different land management strategies on soil quality (Maharjan et al., 2017). In terrestrial ecosystems, such as rangelands, microbial biomass plays a critical role in soil nutrient cycling and quality (Yadav et al., 2011). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) are widely used as indicators of soil carbon and nitrogen dynamics (Maharjan et al., 2017) and nutrient cycling (Liu J. et al., 2011). Furthermore, soil respiration (a key ecosystem process that releases carbon from soil as CO_2) is affected by land management (Wang et al., 2013).

It is already known that any kind of soil disturbance causes significant declines in soil microbial biomass (Kaschuk et al., 2010), suggesting that determination of soil fungal biomass can help to predict the outcome of grazing regimes. Aleixo et al. (2014) demonstrated the importance of soil fungal biomass to understand microbial functionality and nutrient cycling under differing soil management systems. Furthermore, arbuscular mycorrhizal fungi (AMF) are particularly sensitive to soil management variations, so they can be considered good indicators of management quality (Castillo et al., 2006). In this regard, their spore density can determine the ability of AMF to withstand a given grazing regime (Oruru and Njeru, 2016).

The effects of grazing management on the soil biological functions that control rangelands' nutrient cycles are not well documented. On the other hand, the sufficient data from rangelands of moderate climate is not available. Thus, our study aimed to simultaneously evaluate variations of physical, chemical and biological properties of soils under various grazing management regimes (unlimited, managed, and excluded) in north-eastern Iran (North Khorasan Province), to assess the influence of grazing management regime on soil functions. Since grazing intensities vary over the year, we measured the seasonal variations of such properties. The objective was to address the following questions: (i) is soil fertility improved by deposition of dung and urine by livestock? (ii) are soil microbial variables altered by grazing management? (iii) do soil chemical and biological parameters vary seasonally? (iv) are there any significant correlations between soil C and N contents and biological parameters under the different grazing regimes? The findings of the current study were expected to contribute to further understand the effects of long-term grazing management on natural rangelands, and provide informative data to optimize management of these ecosystems.

Materials and methods

Study area: Location and history

This study was carried out in rangeland ecosystems of the Sarigol region in northeast Iran, in North Khorasan Province, on the eastern ridge of the Alborz Mountains, Aladagh (**Supplementary Figure 1**). The geographical location of this region is 36°55′ to 37°8′N and 57°76′ to 57°47′E. The Sarigol region covers 59500 ha including 20000 ha of protected area and 7500 ha of national park. All livestock grazing has been prohibited in the national park since its establishment by the Iranian government 18 years previously. In the protected area, created 47 years ago, livestock grazing is allowed according to a plan (managed grazing). Both the national park and the protected area are managed by the Iranian Department of Environment. In a third area of around 32000 ha, managed by the Iranian Forests, Range and Watershed Management Organization, hunting is banned but free livestock grazing is allowed throughout the year (under intermittent grazing by sheep) (for more details see **Supplementary Table 1**). The rangelands' altitudes of 1400–2940 m, the mean annual precipitation of the region is 273 mm, and the mean temperature is 13°C; the mean of the minimum and maximum temperature is 6.5 and 18°C, respectively. Furthermore, relative humidity is 56% and potential evapotranspiration has been determined around 573.19 mm. In totally, the climate of this region is more affected by subtropical high-pressure systems, Azores high-pressure, western winds, Siberian, and Mediterranean high-pressure systems. The classification of the soil according to the US soil taxonomy classification system is: silty loam, mixed, mesic, Lithic Torriorthents. The rangeland ecosystems of the Sarigol region are dominated by *Artemisia aucheri* and other non-leguminous grasses (for more information see **Supplementary Table 1**).

Soil sampling

In the central part of each of the three areas, sampling sites were defined that had similar physiographic conditions (aspect, slope, elevation). At each site, three parallel transects of 2000 m were defined (500 meters apart) and five quadrats (1 m × 1 m) were established along each transect (400 m apart), resulting in 15 quadrats (replicates) for each grazing management regime. Five soil samples (25 cm × 25 cm × 30 cm) were taken from each quadrat, and then mixed together to produce a single soil sample per quadrat. Soil sampling was carried out in four seasons of 2020 (April, July, November, and February). In each season, 45 soil samples (i.e., 5 soil samples along each transect × 3 transects per grazing management regime × 3 grazing management regimes) were taken to the laboratory in a cooler. To avoid pseudo-replication (see Hurlbert, 1984), the location of each transect was shifted by approximately 50 m each season.

Laboratory analyses

Each soil sample was divided into two portions: one was air-dried and used for chemical analyses; the other was stored at 4°C for study of soil biological activities. Soil bulk density was measured by the clod method (Plaster, 1985). Some of sampled soils were air-dried, grounded and passed through a 2-mm sieve. The electrical conductivity (EC) and pH of the soils were determined using an Orion Ionalyzer Model 125 901 in a 1/2.5 soil/water solution.

Soil organic carbon, total nitrogen and available phosphorus were determined by the Walkley-Black method (Allison, 1975), Kjeldahl acid-digestion (Bremner and Mulvaney, 1982) and Olsen's method (Homer and Pratt, 1961), respectively. Soil potassium was determined by the normal ammonium acetate method (Bower et al., 1952). Total calcium was determined using EDTA titration (Tucker and Kurtz, 1961).

Soil biological parameters were measured on the samples stored at 4°C. Soil microbial basal respiration (BR) was determined by trapping and measuring the evolved CO_2 over a 5 days period at 25°C (Alef and Nannipieri, 1995; Kamali et al., 2020). Substrate-induced respiration (SIR) was determined using glucose (1%) as substrate (Parsapour et al., 2018). The evolved CO_2 was adsorbed in NaOH and measured by HCI titration (Anderson and Domsch, 1978). Microbial biomass C and N (MBC and MBN) were measured by fumigation-extraction (Brookes et al., 1985; Sparling et al., 1990). Soil microbial entropy (MBC: organic C), qCO_2 (BR: MBC), C availability index (BR: SIR), MBC/MBN, and MBN/N were calculated based on the relations between organic C, N, BR, SIR, MBC, and MBN (Kooch et al., 2019). Fungal biomass was determined by the volume of Ergosterol: Ergosterol of soil fungi was soaked with KOH, then extracted with N-hexane in a separating funnel, dried at 40°C in a rotary evaporator and finally dissolved in methanol before being measured at 282 nm by HPLC (Beni et al., 2017).

In each season, soils were sampled from the rhizospheres of *Artemisia aucheri* Boiss, the most dominant species in all the three study areas. To determine the total number of arbuscular mycorrhizal fungi in the soil, a certain quantity of soil sample was air-dried, sieved to 2 mm, and then the number of spores was counted using the Gerdemann and Nicolson (1963) method under a microscope.

Data analysis

One-way analysis of variance (ANOVA) was used to compare soil bulk density, pH, EC, K, P, and Ca between the three grazing management regimes since they were measured on the first soil sampling occasion in spring 2019. The effects of grazing management regime and seasonal factors on those variables, in each season, were tested with two-way ANOVA. Prior to ANOVA, the normality of the variables was checked by the Kolmogorov-Smirnov test, and Levene's test was used to examine the equality of variances. Simple linear correlation and regression analyses were used to characterize the relationships between soil C and N contents and microbial activities across different grazing management types. All statistical analyses were conducted using the SPSS v. 16 statistical software package.

Results

Soil compaction

To understand the impact of livestock grazing on soil compaction, we measured soil bulk density and our statistical analysis indicated that livestock hoof significantly increased the soil bulk density. Bulk density was significantly higher (1.63 g cm^{-3}) under unlimited grazing than under managed grazing (1.41 g cm^{-3}) and grazing-exclusion (1.23 g cm^{-3}) (**Supplementary Figure 2**).

Soil physico-chemical parameters

Soil pH differed significantly ($P < 0.001$) according to the type of grazing management, ranking as unlimited grazing >managed grazing ≈ grazing-excluded, while the soil EC did not differ significantly ($P > 0.05$) (**Supplementary Figure 2**). Although soil phosphorus content was affected by the different grazing regimes, potassium and calcium contents did not vary significantly. Soil phosphorus content was lower under unlimited grazing (15.5 mg kg^{-1}) than under the other two grazing regimes studied (around 25–26 mg kg^{-1}).

Seasonal patterns of soil nitrogen and carbon contents

Soil nitrogen and carbon contents varied significantly in response to grazing management regime (G) and season (S) (**Supplementary Figure 4**). Nevertheless, the interaction S × G had no significant effect on these soil variables. Soils from the unlimited grazing site always had lower carbon contents than those from other regimes. In contrast, the greatest soil nitrogen values were always observed in soils from under unlimited grazing. The soils from sites under managed grazing and grazing exclusion showed similar contents of nitrogen and carbon. Taken separately, the grazing regime and the season led to significant variations of soil C/N ratio, but the S × G interaction did not. Soil C/N ratios were significantly lower under livestock grazing (unlimited and managed) than under grazing exclusion. With regard to the contributions of independent single factors (i.e., grazing management type, season and their interaction) on the variability of C, N, and C/N (see **Supplementary Figure 3**), the season was the principal factor explaining variations in soil nitrogen (51.5%) in the studied rangelands.

Seasonal patterns of soil microbial parameters

Basal respiration (BR) varied significantly through the seasons but grazing management and S × G interaction did not have significant effects (**Supplementary Figure 5**). Under all three grazing management regimes BR was highest (0.7–0.9 mg CO_2 g^{-1} day^{-1}) in summer and lowest in winter (0.1–0.2 mg CO_2 g^{-1} day^{-1}). It ranged between 0.3 and 0.5 mg CO_2 g^{-1} day^{-1} during the spring.

Substrate-induced respiration varied significantly in response to grazing management, to season, and to the interaction between season and grazing management (**Supplementary Figure 5**). SIR was lowest (0.33 mg CO_2 g^{-1} day^{-1}) in winter in soil from the site with unlimited grazing. The highest values were found in the summer under grazing exclusion (2.32 mg CO_2 g^{-1} day^{-1}) and under managed grazing (2.12 mg CO_2 g^{-1} day^{-1}), and in the spring (2.08 mg CO_2 g^{-1} day^{-1}) and autumn (2.02 mg CO_2 g^{-1} day^{-1}) under grazing exclusion.

The single effects of season and grazing management, as well as their combination significantly affected the microbial biomass carbon (MBC). MBC was significantly lower under unlimited grazing than under managed and excluded grazing, with the lowest value (around 30 mg kg^{-1}) in winter. Under unlimited grazing MBC ranged between 70–90 mg kg^{-1} from spring to autumn. The highest MBC content was recorded during summer and autumn in soils under excluded (246–248 mg kg^{-1}) and managed grazing (219–225 mg kg^{-1}). In the grazing-excluded and managed grazing sites, the lowest soil MBC (around 140 mg kg^{-1}) was measured in winter. The interaction of grazing management and season had a significant impact on microbial biomass nitrogen (MBN) although the effect of each factor alone was not significant. The greatest MBN contents were observed in soils from excluded (6.98 mg kg^{-1}) and managed grazing (6.93 mg kg^{-1}) rangelands. The lowest MBN values were obtained from soils of the unlimited grazing site, in spring (2.76 mg kg^{-1}) and winter (3.07 mg kg^{-1}). MBN was higher in summer under all the grazing management regimes.

Considering the contribution of independent single factors (i.e., grazing management type, season, and G × S) to the variability of the studied soil microbial parameters (see **Supplementary Figure 3**), the season was the principal factor explaining variation in basal respiration (BR) (62.99%), while grazing management regime determined variations in SIR (44.3%) and MBC (61%).

Seasonal patterns of soil fungal biomass and AFM spore density around roots of *Artemisia aucheri*

Both season and grazing management had significant impacts on soil fungal biomass, although their combination had no significant effect on this soil variable (**Supplementary Figure 6**). Fungal biomass in the soils was the lowest under unlimited grazing (always below 1 mg kg^{-1}), and the highest in grazing-excluded sites. Under grazing exclusion and unlimited grazing, fungal biomass was significantly lower in winter than in the other seasons.

The two factors (season and grazing management) and their interaction (S × G) had significant effects on AFM spore density around roots of *A. aucheri* (**Supplementary Figure 6**). This was significantly higher in summer in soils from rangelands under unlimited grazing (73.93 spores per g soil) and grazing exclusion (73.46), and also in autumn under unlimited grazing (72.6). The lowest AFM spore density was measured during the winter in all grazing (25–35 spores per g soil). Generally, the soils around roots of *A. aucheri* under unlimited grazing had higher AFM spore densities than soils under the other grazing regimes. Considering the contribution of independent single factors (i.e., grazing management type, season, and G × S) to the variability of soil fungal parameters (see **Supplementary Figure 3**), the grazing regime determined most changes in fungal biomass (63.35%), whereas season explained most of the variability of AFM spore density (45%).

Seasonal patterns of some soil stoichiometric microbial indicators

All the studied stoichiometric indicators except the microbial metabolic quotient were affected by season and grazing regime, and by their combination (S × G) (**Supplementary Table 2**). Mean CIA ranged from 0.11 (under managed grazing) to 0.83 (under unlimited grazing) and both minimum and maximum values were recorded in winter. Across seasons, CIA was significantly lower under grazing exclusion (0.13–0.35) than under unlimited and managed grazing, and greatest under unlimited grazing (0.39–0.83), followed by managed grazing (0.11–0.45). Microbial entropy was always lower (1–2.4) under unlimited grazing than managed grazing (3.2–4.7) and grazing exclusion; this indicator was greatest during autumn under all grazing regimes.

During summer, qCO_2 was highest under unlimited grazing (0.10) and lower (0.01) under managed grazing and grazing exclusion. Generally, qCO_2 was higher under unlimited grazing than the two other regimes, and highest in summer.

The soil MBC/MBN ratio was highest in autumn under grazing exclusion (around 60) and lowest in winter under unlimited grazing (around 10). Under all grazing regimes, MBC/MBN was lowest in winter. Generally, MBC/MBN was highest in soils under grazing exclusion.

As with MBC/MBN, the MBN/N ratio was lowest in spring under unlimited grazing (0.39), and highest in winter under grazing exclusion (1.65) and managed grazing (1.50). MBN/N was always highest under grazing exclusion. With regard to the contribution of the independent single factors (i.e., grazing management type, season, and G × S) on the variability of the studied stoichiometric indicators (see **Supplementary Figure 3**), the grazing regime was the principal factor explaining variation in qCO2 (42.65%) and microbial metabolic quotient (43.23%).

Correlation and regression analyses of biological variables with organic carbon and nitrogen contents

Based on Pearson correlation and regression analyses (**Supplementary Figure 7**), significant correlations were found between organic C and SIR ($r = 0.54$, $F = 76.53$, p-value < 0.0001), MBC ($r = 0.59$, $F = 96.39$, p-value < 0.0001), and fungal biomass ($r = 0.56$, $F = 82.03$, p-value < 0.0001). Organic C also showed a significant but low correlation ($r < 0.50$) with BR ($r = 0.29$, $F = 16.80$, p-value < 0.0001) and MBN ($r = 0.46$, $F = 48.64$, p-value < 0.0001). Pearson correlation and regression analyses between the above variables with soil nitrogen were also carried out (data not shown), and a high and significant correlation was only observed for BR ($r = 0.526$, $F = 66.11$, p-value < 0.0001).

Discussion

In the Sarigol region, unlimited and managed grazing by livestock during 47 years reduced plant cover by 30 and 15%, respectively, compared with sites where the presence of sheep has been forbidden for 18 years. It has frequently been reported that plant cover in rangeland ecosystems strongly affects soil functions and ecosystem health, and that the performance of soil microorganisms is directly related to plant cover and litters (Teague et al., 2011). Therefore, it seemed important to determine and compare the effects of grazing and trampling by livestock on soil physical, chemical and microbial characteristics of these rangelands under three different grazing management regimes.

The bulk density of the soil under unlimited grazing was greatest; its soil was around 25% more compacted than that under grazing exclusion. Also, managed grazing led to more soil compaction (around 13%) than grazing exclusion, due to the pressure of livestock hooves. Our results were in line with those of Binkley et al. (2003) and Hashemi et al. (2019), who reported higher soil bulk densities after trampling by animals. It can be stated that one of the visible impact of unlimited livestock grazing in rangelands is soil compaction that subsequently causes further effects on the soil functions.

The levels of nutrients P, K, and Ca in soils under the three grazing regimes were compared. Although soil K and Ca contents did not differ between grazing regimes, the P content was sharply lower under unlimited grazing. After 47 years of unlimited grazing, soil P content was 40% lower than in the site under grazing exclusion for 18 years. In line with other investigations (Jaafari et al., 2014; Hashemi et al., 2019), our study reports that P content is sensitive to soil compaction. Decreasing litter accumulation in the unlimited grazing fields has probably caused the decline of soil P concentration, as proposed by El-Dewiny et al. (2006).

There was no significant variation in electrical conductivity in response to the studied grazing regimes. Nevertheless, soil pH was slightly higher under unlimited grazing (7.5 vs. 7.2–7.3). In agreement with the findings of Xie and Wittig (2004) and Liebig et al. (2006), this confirms that livestock grazing has no negative impact on soil pH and EC. Although livestock excreta contain some salt that can increase soil salinity (Ajorlo et al., 2011), the EC of the soil was stable and variations of pH were low under all grazing regimes.

Bolan et al. (2004) believed that the fertility of rangeland soils is substantially affected by the deposition of dung and urine, and their subsequent transformation in soil. Zarekia et al. (2012) found that livestock manure is a nitrogen input to soil. The data presented here clearly show that in all seasons, especially spring, soil N content was greater under unlimited grazing than under grazing exclusion and managed grazing, so the presence of livestock on the rangelands improved soil N content. In contrast to nitrogen, soil organic C content was 3–4.5% under unlimited grazing, lower than under managed grazing (4–6.5%) and grazing exclusion (5–6.5%). In line with our finding, Ganieva et al., 2019 reported a 6.07% decrease in organic carbon content in the soils of dry steppes of Azerbaijan. The lower concentration of soil organic C under unlimited grazing was probably due to decreased vegetation cover and litter (Yong-Zhong et al., 2005).

In the spring and summer, when the grazing intensity is higher, the soil N level is higher and C is at a minimum. However, dropping of N and C content to the minimum in the winter was expectable. In this study, the C/N ratio was >7.5 in soils under unlimited grazing, and 8–14.5 under managed grazing and grazing exclusion. Although soil C/N ratio is strongly driven by plant cover (Lucas-Borja et al., 2011), it is substantially decreased by addition of nitrogen from livestock urine. In agreement with our study, Qasim et al. (2017) demonstrated that unmanaged grazing severely affected vegetation coverage, soil organic matter, large-sized soil aggregates, nitrogen mineralization and soil moisture contents of arid shrubland of Baluchistan, Pakistan.

Significant differences in soil basal microbial respiration in response to livestock grazing regime were not recorded. Variations were largely seasonal (see **Supplementary Figure 2**), with a maximum always in spring, maybe because soil respiration depends on precipitation and soil temperature (Zarafshar et al., 2020). Besides, soil biological activities such as microbial respiration are strongly influenced by season through changes in biotic and abiotic factors (Kaiser et al., 2010; Bagheri Delijani et al., 2022).

The highest values of SIR were measured during summer and spring, and were lowest under unlimited grazing. Parallel to this study, Bagchi et al. (2017) also observed adverse effects of continuous grazing on soil microbial respiration. Sankaran and Augustine (2004) hypothesized that the intensity of grazing (percentage of aboveground production consumed) is an important factor regulating soil microbial activity. This study indicated that plant cover changes in a rangeland ecosystem can cause changes in the nitrogen and carbon of microbial biomass (MBC and MBN), since lands with lower plant cover, under unlimited grazing, had higher MBC and MBN than those with higher plant cover (manage grazing and grazing exclusion). The decline in soil MBC and MBN in response to unlimited grazing was probably due to the decrease of carbon input from litter decomposition (Zarafshar et al., 2020). Our analyses indicated a significant relation between organic C and MBC and MBN. MBC and MBN were mostly highest in the spring under all grazing regimes. In accordance with our finding, low MBN under continuous grazing in northern Iran has been reported (Kooch et al., 2020).

In line with Taddese et al. (2007), we observed that soil fungal biomass declined to about a third in response to unlimited grazing when compared with grazing exclusion or even managed grazing. According to Goomaral et al. (2019), shifts in soil fungal communities caused by livestock grazing are related with changes in the biomass and diversity of functional vegetation groups. Our floristic data (see **Supplementary Table 1**) demonstrate that the plant community and frequency of the Sarigol region has been shifted due to unlimited grazing, suggesting that the decrease in fungal biomass can be explained by the plant community change. Surprisingly, the density of AMF spores in the rhizosphere of *A. voucher* plants under unlimited grazing was higher than that under managed grazing. Not only was AMF spore density higher under unlimited grazing than under grazing exclusion in autumn, but similar AFM spore densities were recorded in other seasons. We hypothesize that the N fertilizer effect of livestock urine is countered by stressful biotic and abiotic conditions induced by long-term grazing, leading to greater AMF symbiosis with roots of *A. voucher*. In contrast to the results presented here, many investigators [Egerton-Warburton and Allen (2000), Bhadalung et al. (2005), Mbuthia et al. (2015)] found that the use of nitrogen fertilizers caused a decrease in AMF spore density.

In this study, the highest microbial metabolic quotient was detected under unlimited grazing, illustrating a lower efficiency of microbial activities in the soil (Fterich et al., 2014), supporting the findings of Kooch et al. (2020), who reported higher qCO_2 under continuous grazing.

Moreover, our finding is supported by other investigators who reported that non-grazing rangelands show higher soil microbial entropy (Juan et al., 2008; da Silva et al., 2012). In this regard, we clearly observed lower soil microbial entropy under unlimited grazing than under grazing exclusion and managed grazing. In all, the greatest values of the C availability index or CIR (BR $<$ SIR) were found in soils under unlimited grazing, indicating that there were no large differences between BR and SIR values, while more disturbed soil showed BR $>$ SIR (Zarafshar et al., 2020). SIR can indicate fungal, bacterial, and total microbial responses to glucose-induced respiration, as well as the potentially active microbial biomass present in decomposing plant residues (Beare et al., 1990), so it can be concluded that the microbial community was degraded under unlimited grazing. As we had speculated, lower values of MBC/MBN and C/N were detected in rangelands under higher grazing intensities.

Conclusion

Our study demonstrated that soil organic carbon and phosphorus contents are sensitive to differently managed rangeland ecosystems and can be considered as promising indices to evaluate grazing management regimes. Our data indicate that soil microbial functions drastically declined as a result of removal of plant cover by grazing. In this regard, unlimited grazing had the most deleterious impact on microbial structures, specifically fungal biomass. This study found evidence that managed grazing had a less detrimental impact on soil microbial variables (microbial respiration and biomass) than unlimited grazing, mostly due to higher input of fresh plant residues into the soil. In conclusion. Nevertheless, implementation of intermittent grazing to maintain soil health is strongly recommended. Moreover, the assessment of some soil services like carbon and nitrogen stocks under different graze management regimes could provide a set of detailed information for better management.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AS and NK conceived and planned the experiments. MB carried out the experiments. MZ and SB contributed to the interpretation of the results. MZ took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2023.1028149/full#supplementary-material

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