

Original Research

Microbial Properties and Dehydrogenase Activity in Semiarid Area, Kerman Province, Iran

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Abstract

Soil microbial and biochemical properties are important indicators in estimating soil degradation. This study aimed to determine the impact of site, season, and grazing on microbial population size, microbial biomass carbon, and dehydrogenase activity in two semiarid areas in Kerman Province, Iran. Soil samples were taken from four sites that were either cold or warm and either grazed or not-grazed over two seasons. Microbial count, microbial biomass carbon, and dehydrogenase activity were determined via plate count, fumigation-extraction, and colorimetric methods, respectively. The statistical analysis showed a significant difference in fungal and bacterial plate count, microbial biomass, and dehydrogenase activity among the studied areas. Fungal and bacterial counts, microbial biomass carbon, and dehydrogenase activity were greater at cold sites compared to warm sites, possibly due to increased plant cover, soil organic carbon, and moisture. In addition, there was temporal variation in microbial properties and dehydrogenase activity with the increased activity of the spring samples. Grazing did not change the soil chemical properties but did alter its microbiological properties and dehydrogenase activity, indicating their greater sensitivity in estimating soil quality. Based on the measured microbial properties, the cold sites have an advantage in sustaining soil quality, and the warm sites are more likely to require protective management programs.

Keywords: bacteria, dehydrogenase, fungi, organic carbon, semiarid

Introduction

“Land degradation” is a composite term referring to a loss in productivity and land resources, such as soil, water, and biodiversity [1-2]. The term is often used interchangeably with “soil degradation,” and the two are closely linked because soil degradation processes

constitute the most significant land-degradation processes. Degraded soil must be understood as the failure of all or some of the soil functions [3], such as the filtration of water [4] and retaining vegetation [5].

Estimating soil quality or degradation depends on a large number of physical, chemical, biological, and biochemical properties. It is very important to choose indicators that respond rapidly to changes in environmental factors. The microbiological and biochemical properties of soils are known to be very sensitive

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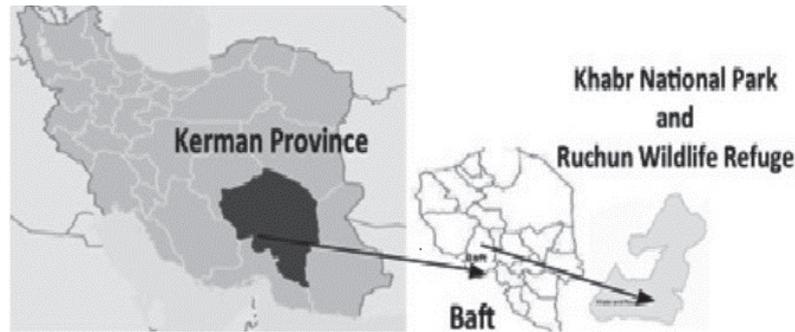


Fig. 1. Khabr National Park and Ruchun Wildlife Refuge in Baft, Kerman province, Iran.

indicators of soil quality or degradation [6-7]. There is a direct relationship between soil microbiological and biochemical properties, and that soil function and sustainability, and suitable microbial activity is required to maintain soil quality.

Iran, with an area of 1.64 million square kilometers, is located in the arid and semi-arid zone. Approximately 73% of the country has an arid and semiarid climate [8], which is vulnerable to land degradation and, in consequence, desertification due to the increasing population pressure on the land due to grazing and the consumption of water resources [9]. While the quality of a soil is related to its physical, chemical, and biological properties, only physical processes and the biology of soil flora and fauna have been relatively well studied in the semiarid areas of Iran [10-14]. Knowledge about soil microbial properties remains unclear and fragmentary in these semiarid areas [15-16]. The purpose of this study was to determine and compare the effects of site, season, and grazing on microbial population size (bacteria and fungi), microbial biomass carbon (MBC), and dehydrogenase activity, as a general index for microbial

activity, over two seasons in two semiarid areas located in Khabr National Park and Ruchun Wildlife Refuge, Kerman province, Iran.

Material and Methods

Study Area and Soil Sampling

The studied area is located in the center of Iran within the semiarid steppe region of Khabr National Park and Ruchun Wildlife Refuge (Fig. 1). This national park extends from 28°28' to 28°58' N and from 56°02' to 56°38' E. The mean annual temperature ranges between 17.5 and 21.0°C, and precipitation ranges between 200 and 350 mm per year. Initially, two plots in cold sites and two plots in warm sites were selected. The altitude of the warm sites is 1,707 m a.s.l and is dominated by *Artemisia siberi*. The cold sites have an altitude of 2,365 m a.s.l. The ecosystem at the cold sites is dominated by *Stipa hassknechti* and *A. siberi*. At both the cold and warm sites, grazed and not-grazed areas were

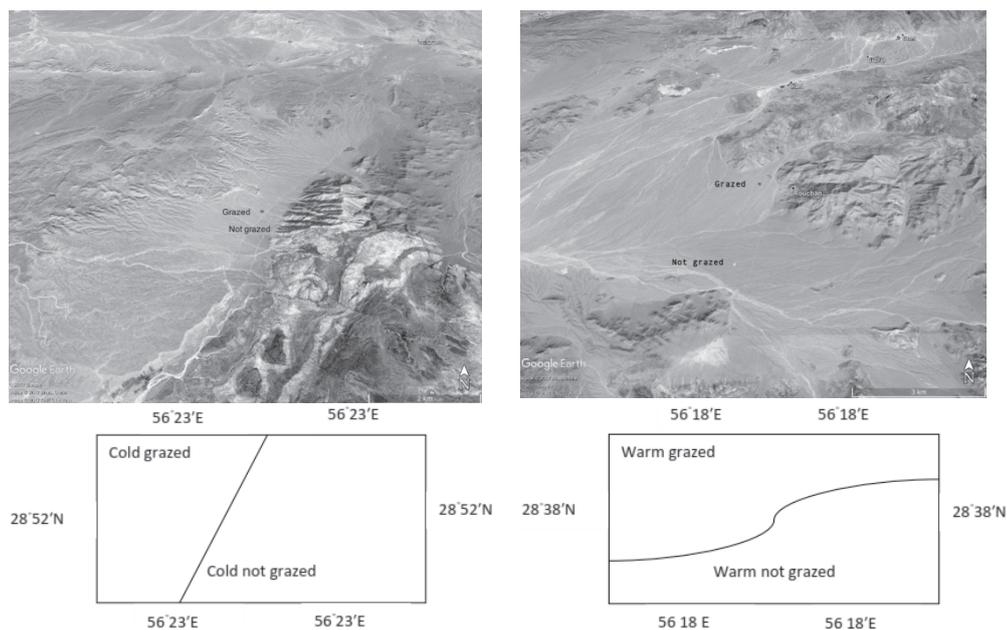


Fig. 2. Precise location of grazed and not-grazed areas in cold sites (left) and warm sites (right).

Table 1. Mean comparison among site, season and grazing treatments.

Chemical Properties	Site		Season		Grazing	
	Cold	Warm	Autumn	Spring	Not-grazed	Grazed
pH 1:2.5 water soluble	7.75 ^a	7.85 ^a	8.3 ^b	7.9 ^a	8.75 ^a	7.852 ^a
Electrical Conductivity	99.66 ^a	100.57 ^a	99.62 ^a	100.26 ^a	100.02 ^b	99.85 ^a
Total Nitrogen (%)	0.083 ^a	0.051 ^b	0.071 ^a	0.062 ^b	0.074 ^a	0.078 ^a
Available Phosphorous (mg/kg)	35 ^a	35.05 ^b	31 ^b	34 ^a	33.1 ^a	34.2 ^a
Extractable Potassium (mg/kg)	237.59 ^a	183.44 ^b	223.92 ^a	202.72 ^b	220.35 ^a	224.11 ^a
Soil Organic Carbon (%)	0.65 ^a	0.29 ^b	0.39 ^a	0.45 ^a	0.51 ^a	0.55 ^a
Soil Organic Matter (%)	1.11 ^a	0.49 ^b	0.67 ^a	0.77 ^a	0.87 ^a	0.94 ^a
Soil Moisture (%)	4.44 ^a	1.32 ^b	4.83 ^a	0.93 ^b	3.02 ^a	3.12 ^a

Soil organic matter (%) calculated by soil organic C% \times 1.72. Different letters show significant difference determined by LSD test ($p < 0.05$).

selected (Fig. 2). Thus, in the analysis, four treatments were considered as follows: (1) cold grazed (CG), (2) cold not-grazed (CNG), (3) warm grazed (WG), and (4) warm not-grazed (WNG).

Soil sampling was performed from a plot area of 100 \times 100 m for the four mentioned treatments in the spring and autumn. Eight composite soil samples were collected from the top 10 cm of soil for each treatment, sieved (2 mm mesh) to remove plant tissues, and kept at 4°C until further analysis. The soil texture was sandy loam with an average of 10% clay, 30% silt, and 60% sand. The soil's chemical properties (pH, electrical conductivity, total nitrogen, available phosphorous, extractable potassium, soil organic carbon, soil organic matter, and soil moisture) were measured according to standards methods of soil analysis.

Bacteria and Fungi Count

The plate count method was used to estimate the number of aerobic heterotrophic bacteria and filamentous fungi. Ten grams of fresh soil sample were added to 90 ml of 0.9% (w/v) sodium chloride solution. After homogenization for 30 min, this solution was decimally diluted (10^{-1} to 10^{-7}), and 0.1 ml of the resulting solution was plated on suitable media and spread uniformly. Nutrient Agar (MERCK) and Saburo Dextrose Agar (MERCK) were used for culturing aerobic heterotrophic bacteria and filamentous fungi, respectively. The incubation time for bacteria and fungi was 3 and 4 days, respectively. Each dilution was plated in duplicate, and the population was expressed as the number of colony-forming units (CFUs) per 1 g of oven-dried soil [17].

Microbial Biomass Carbon

The microbial biomass carbon (MBC) was estimated via the chloroform fumigation-extraction method. The fumigated and non-fumigated soils (equivalent to 10 g of oven-dried soil) were extracted with K_2SO_4

(0.5 M) solution and filtered. The MBC in the extract was determined via the wet oxidation-titration method [18].

Dehydrogenase Activity

Dehydrogenase activity was determined based on a method described by Schinner et al. [19]. Briefly, 5 g of moist soil were weighed in glass vials and treated with 2.5 ml of 1% triphenyltetrazolium chloride (TTC)-Tris buffer. The suspensions were then incubated in the dark at 25°C for 16 h. After incubation, the triphenylformazan (TPF) was extracted with acetone and estimated colorimetrically. All measurements were carried out in triplicate with one blank.

Statistical Analysis

The measured properties were subjected to analysis of variance (ANOVA), and the means were compared via the least significant difference (LSD) test ($p < 0.05$).

Results and Discussion

In this study, soil variables, including chemical properties, microbial count, microbial biomass carbon and dehydrogenase activity, were measured to determine the effects of site, season and grazing. There were significant effect on the part of site and season on soil chemical and microbial properties and dehydrogenase activity. Grazing affected soil microbial properties and dehydrogenase activity, but not soil chemical properties.

Soil Chemical Properties

Table 1 summarizes the chemical properties of the soils. There were significant effects on the part of site and season on soil chemical properties. Although the values

of the soil chemical properties were higher in the grazed area as compared to the not-grazed area, the observed differences were not statistically significant (Table 1).

Soil pH and electrical conductivity (EC) are the principal indicators of the chemical characteristics of a particular soil, playing a significant role in soil biogeochemical processes, the solubility of soil nutrients, plant growth, and microbial and enzyme activity in the soil [20-21]. Soil showed alkaline reaction, which are normally found in arid/semiarid regions because little leaching and high evaporation causes ions to concentrate in the soil. Our results indicate that pH and electrical conductivity did not change easily due to site and grazing effects, as is supported by other studies [22-23]. The low pH values of the spring samples may be explained by production of CO₂ via more active plant roots and bacteria, which can temporarily lower the pH value of natural soils [24-25].

Total nitrogen (TN), available phosphorous (AP), extractable potassium (EK), soil organic carbon (SOC), and soil organic matter (SOM) are used as important indicators of soil quality [26-27]. Regardless of season, at the cold sites, the values of TN, AP, EK, SOC, SOM, and soil moisture were higher than at the warm sites (Table 1). The higher TN, AP, EK, SOC, and SOM values at the cold sites (Table 1) may be attributed to their increased plant cover, which adds nutrients to the soil. In addition, there is some evidence that plant cover decreases nutrient loss from the soil as well [28]. The soil organic carbon threshold for sustaining soil quality is widely suggested to be about 2%, below which potentially a serious decline in soil quality will occur [29]. The studied sites had low soil quality based on their SOC content (less than 2%). The lower SOC values at warm sites as compared to cold sites likely result from the faster turnover of organic carbon and the negative correlation between temperature and soil organic carbon [30].

There was no temporal variation in SOC, but TN, AP, and EK changed seasonally (Table 1). The temporal alteration of AP occurred differently than that of total nitrogen and EK. Plants uptake more phosphorous with increasing temperature [31], thus depleting the available phosphorus in the soil from spring to autumn, which is in accordance with our results. In the spring, the observed reductions in the amounts of TN and EK may be related to the higher demands on the part of the soil biological community due to its metabolic activity.

In addition, the higher levels of TN seen in autumn can be related to the increased activity of nitrogen fixing bacteria [32]. The observed seasonal differences in soil nutrients are due to changes in soil pH, moisture, and temperature as well [33].

Soil Microbial Population Count

Soil microbial communities are primarily composed of bacteria and fungi. Changes in soil bacteria and fungi are expected to affect soil fertility and productivity [34]. The effects of site, season and grazing were significant ($p < 0.05$) on both bacterial and fungal counts. The bacterial CFUs/g soil ranged from 5.68×10^5 at cold sites to 2.73×10^4 at warm sites, from 5.25×10^5 in spring samples to 4.30×10^5 in autumn samples, and from 2.66×10^5 at not-grazed areas to 3.27×10^5 at grazed areas (Table 2). Regardless of season and grazing, increased bacterial counts were detected in the soil from the cold sites (Table 2). The fungal CFUs/g soil varied from 5.43×10^3 at the cold sites to 2.33×10^3 at the warm sites, from 3.45×10^3 in spring samples to 2.55×10^3 in autumn samples, and from 3.35×10^3 in not-grazed areas to 4.41×10^3 in grazed areas (Table 2). This value was increased for cold sites, spring samples, and not-grazed areas as compared to warm sites, autumn samples, and not-grazed areas, respectively. The increased microbial population size at cold sites may be related to their increased organic matter, plant cover, and moisture (Table 1), which all affect microbial growth [35-36]. Our findings regarding increased bacterial counts in not-grazed areas are supported by Anguita et al. [37]. However, some reports stand in contrast with our findings. Increased bacterial and fungal counts have been reported in intensively grazed areas, indicating that intensive grazing favors the proliferation of bacteria and fungi due to the increase of organic matter caused by animal excreta [38-39]. The lower fungal CFUs/g values seen in our study may be explained by the higher soil pH (Table 1), which is not favorable for fungal growth [39].

Microbial Biomass Carbon

Soil microbial biomass (MBC) plays a crucial role in nutrient cycling and is a sensitive indicator of the dynamics of the soil C and N cycles. Among the microbiological indicators of soil quality, microbial

Table 2. Mean comparison among sites, sampling times, and grazing on soil microbial properties.

Microbial Properties	Site		Season		Grazing	
	Cold	Warm	Spring	Autumn	Grazed	Not-Grazed
Bacterial Count (CFU/g soil)	5.68×10^5 ^a	2.55×10^4 ^b	5.25×10^5 ^a	4.3×10^5 ^b	2.66×10^5 ^b	3.27×10^5 ^a
Fungal Count (CFU/g soil)	5.43×10^3 ^a	2.33×10^3 ^b	3.45×10^3 ^a	2.55×10^3 ^b	3.35×10^3 ^b	4.41×10^3 ^a
Microbial Biomass Carbon ($\mu\text{g/g}$ soil)	713.98 ^a	516.96 ^b	645.12 ^a	522.15 ^b	755.519 ^a	475.44 ^b

Different letters show significant difference determined by LSD test at $P < 0.05$ level.

biomass carbon (MBC) is one of the most promising and most commonly used due to its higher sensitivity as compared to physical and chemical properties, including soil organic carbon [40-41]. In addition, soil microbial biomass is an important indicator of soil fertility in soil ecological studies and sustainable environmental management [42-43].

The analysis of the microbial biomass carbon (MBC) data showed the significant effects on the part of site, season, and grazing on MBC value. MBC value was significantly higher for cold sites, spring samples and grazed areas than for warm sites, autumn samples, and not-grazed areas, respectively (Table 2). The higher MBC values at the cold sites represent an advantage in terms of maintaining soil quality. Thus, it has been proved that MBC values are affected by various environmental parameters. The effects of organic matter, temperature, moisture content, and pH on soil microbial biomass value have been reported [44-45]. The increased microbial biomass at cold sites can be explained by their increased plant cover, resulting in the increased accumulation of litter and fine roots at the cold sites as compared to warm sites. Many studies indicate the effect of plant cover density and plant diversity on MBC [46]. The increased organic matter inputs from plant litter and root exudates may have enhanced the rate of MBC production at the cold sites via the improved growth of microbial populations and the accumulation of C in the microbial biomass [47]. In addition, the positive correlations between total nitrogen and soil organic C and MBC have been proven in many studies [35-36, 48], which could explain the increased MBC at the cold sites as well (Table 1). Soil moisture is another important factor in microbial biomass values in soil [49-50]. The increased MBC observed in the soils taken from the cold sites may be related to the increased moisture at the cold sites (Table 1). Although some studies that have found no significant relationship between soil water content and microbial biomass carbon [51-52], other studies are in agreement with our findings, indicating increased MBC values as soil water content increases [53-54]. We also found increased MBC values in grazed areas, potentially due to the addition of organic matter via animal excreta and thus improved microbial growth. The soil microbial biomass response to grazing by livestock or other large animals is not constant and has been reported to increase or decrease in response to grazing [55]. Increased MBC values have been reported in areas with low grazing intensity [35], which supports our findings. It seems the effect of grazing depends on grazing intensity. Heavy grazing destroys the soil structure, disturbing microbial growth and the metabolism of microorganisms and thus decreasing MBC values [56-58].

Dehydrogenase Activity (DHA)

Dehydrogenases are intracellular enzymes involved in the oxidative processes of viable microbial cells. Therefore, their activity is considered a measure of overall

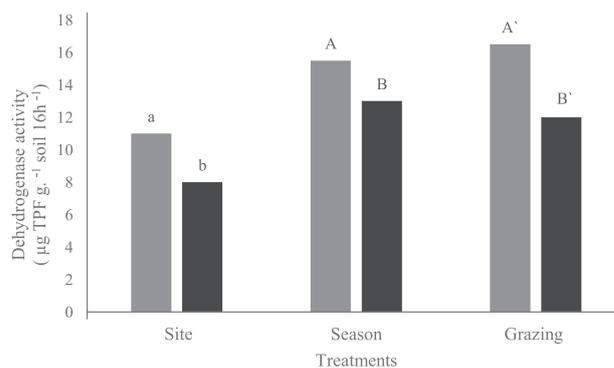


Fig. 3. Dehydrogenase activity in soil of different sites during different seasons and under grazing treatment; different letters in each treatment show significant differences determined by LSD test at $p < .05$ level; a, A, A' indicate more dehydrogenase activity at the cold site, spring samples, and the grazed area as compared to warm sites (b), autumn samples (B), and the not-grazed area (B'), respectively.

soil microbial activity and microbiological quality of soils [59-60]. Generally, these enzyme activities in the soil are closely related to organic matter content in the soil [61-62]. Our results indicated more dehydrogenase activity for the cold sites, spring samples, and grazed areas as compared to the warm sites, autumn samples, and not-grazed areas (Table 2 and Fig. 3). Generally, the enzyme activities in the soil were closely related to organic matter content, indicating greater biological activity in the soil and the stabilization of extracellular enzymes through complexation with humic substances [63]. The increased dehydrogenase activity at the cold sites may be explained by the increased organic matter and moisture (Table 1) at the cold sites, which is in agreement with other studies [64-66].

Variation in dehydrogenase activity was observed, with increased dehydrogenase activity being seen in the spring samples. Seasonal changes in enzyme activities are not entirely understood and depend on numerous factors, such as aeration, soil moisture, soil temperature, flora, and microflora [67]. Seasonal variation in dehydrogenase activity has been reported by others [68-69], which is in agreement with our findings. Higher levels of dehydrogenase activity in the spring is due to the better environmental conditions for microbial activity, especially temperature and moisture, in the spring, favoring the active proliferation of microbes as compared to autumn [70].

There was more dehydrogenase activity in the grazed areas as compared to the not-grazed areas (Table 2) due to the increased microbial biomass in the grazed area [50]. There are inconsistent results regarding the effect of grazing on dehydrogenase activity. Reductions of dehydrogenase activity have been reported with increased stocking rates in arid grazed areas [71-72], while others have reported increases or no change in dehydrogenase activity in the grazed area [73]. It seems that grazing intensity determines grazing effect on dehydrogenase activity.

Conclusion

We have concluded that microbial biomass, the abundance of bacteria and fungi, and dehydrogenase activity in the surface soil were strongly influenced by various environmental factors, including organic matter, moisture, and plant cover. Grazing affected only soil microbial properties, indicating the greater sensitivity of these properties when used in soil monitoring. Based on the chemical and microbial properties and dehydrogenase activity, the quality of the soil at the warm sites is lower, and these warm sites are at risk of losing their function and becoming degraded. The warm sites require more consideration with regard to conservation programs.

Conflict of Interest

The authors declare no conflict of interest.

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