# ASSESSING SOIL QUALITY THROUGH SOIL CHEMICAL PROPERTIES AND ENZYME ACTIVITIES IN SEMIARID AREA, IRAN

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Abstract. Semiarid ecosystems are more sensitive to environmental changes than other terrestrial ecosystems, which makes their monitoring very important. Determining soil quality is critical to precise monitoring of semiarid area, and its evaluation involves the assessment of soil properties. In this study, the soil chemical properties and enzyme activities were measured and compared in order to determine soil quality. Four plots were chosen in the cold and warm sites in the Khabr national park and Ruchun wildlife refuge with grazed and not grazed areas. Samples of surface soil (0-10 cm) were collected in spring and autumn. The results showed that chemical properties of the soils were significantly affected by site (soil organic carbon, total nitrogen, available phosphorus and soil moisture) and season (pH, total nitrogen, available phosphorus and soil moisture) but not by grazing. All assayed enzyme activities were significantly influenced by site and season. Alkaline phosphatase activity was affected by grazing, too. Soil chemical properties (soil organic carbon, total nitrogen, and available phosphorus content and soil moisture) and enzyme activities (acid phosphatase, alkaline phosphatase, invertase,  $\beta$ -glucosidase, urease and arylsulfatase) showed higher value at cold sites. The positive correlation between all assayed enzymes and soil organic carbon (r= 0.189-0.639) indicated the important role of soil organic carbon availability in soil enzyme activities. Seasonal variation was observed in soil chemical properties except for electrical conductivity. In addition, temporal variation was observed in enzyme activities with more activity in spring samples except for arylsulfatase activity. According to more value in nutrient content and enzyme activities, it can be concluded that soil at cold sites have higher quality than warm sites. In conclusion, the warm sites with poor soil quality need more concern to be protected. Keywords: grazing, monitoring, seasonal, semiarid, soil quality

### Introduction

Semiarid ecosystems approximately cover 40% of the Earth's land and are increasing in proportion due to global warming and consequent desertification. The soils, in these ecosystems, characteristically contain less organic matter and plant biomass (Reyes-Reyes, 2007). The fragility of soil in semiarid environments, caused by low organic matter content, lack of vegetation exposes soil to wind and soil erosion, and salty crusts impede root growth, makes soil quality monitoring and management imperative. Various physical, chemical, biological and biochemical indicators have been used to estimate soil quality. It is important to take into account sensitivity to soil management in a wide range, sampling error, cost of measurement and required time. The measurement of soil enzyme activities provides an early indication of changes in soil quality, as they are involved in the mineralization of nutrients such as N, P and C (Trasar-Cepeda et al., 2008). Soil enzymes participate in almost every transformation process of decomposition and play a central role in maintaining soil fertility by releasing mineral nutrients from complex organic resources (Baldrian and Stursová, 2010). Since soil enzyme activities are linked with several ecosystem processes and exhibit rapid response to both natural and anthropogenic disturbances, they have been adopted as a suitable indicators for soil quality (Das and Varma, 2010).

Iran, with an area of 164 million hectares, is located in the mid-latitude belt of arid and semiarid regions of the Earth. The arid and semiarid regions cover more than 60% of the country, 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 (Amiraslani and Dragovich, 2011). The Khabr National Park and the Ruchun Wildlife Refuge is located in the southeast of Iran with various ecological systems and different habitats for plants and animals (Najmizadeh and Yavari, 2006). While the quality of a soil is related to its physical, chemical and biological properties, only several studies has been conducted on physical processes, chemical properties and the biology of soil flora and fauna in the semiarid areas of Iran (Bagheri et al., 2009; Rajabi et al., 2011; Shirvani, 2012; Sharafatmandrad et al., 2014; Mirzaei et al., 2017). There is very little information on soil biological indicators in the Iranian semiarid areas (Raiesi and Riahi, 2014; Kabiri et al., 2016). Indeed, there has not been any study on soil enzyme activities in the Khabr National Park and the Ruchun Wildlife Refuge. Therefore, this study addresses the information gap that exists on the effects of site, season and grazing on soil enzyme activities as important soil quality indicators in this park, which is needed to aide in the overall quality assessment of the ecosystems contained within this park. It was hypothesized that soil chemical properties and enzyme activities are different at cold and warm sites and is also affected by season and grazing. For this purpose, the variation in soil chemical properties and enzyme activities was studied of selected cold and warm sites, which were either grazed or not grazed in spring and autumn seasons.

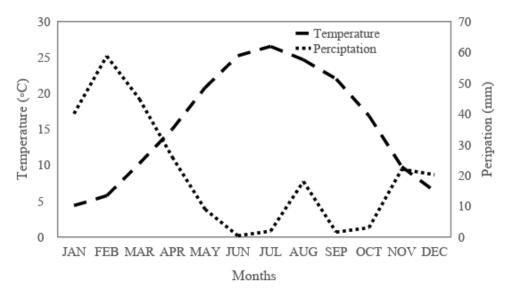
# Materials and methods

# Field site, experiment design and soil sampling

The study area is located in the southeast of Iran within the semiarid steppe region of Khabr National Park and Ruchun Wildlife Refuge, Kerman province, Iran. This park extends from  $28^{\circ}28'$  to  $28^{\circ}58'$  N and from  $56^{\circ}02'$  to  $56^{\circ}38'$  E. The mean annual temperature and precipitation varies between  $17.5-21.0^{\circ}$ C and 200-350 mm, respectively. Mean monthly temperature and precipitation has been given in *Figure 1*. Two plots at the cold sites and two plots at the warm sites were selected as sampling plots for this study. The altitude of the warm site is 1,707 m above sea level (a.s.l) and the vegetation is dominated by *Artemisia siberi*. The cold sites have an altitude of 2,365 m (a.s.l), and the vegetation is dominated by *Stipa hassknechti* and *A. siberi*. At both the cold (C) and warm (W) sites, grazed (G) and not grazed (NG) areas were 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).

Eight soil samples were collected from the top 10 cm of soil from a plot area  $100 \times 100$  m in mentioned treatments in spring (S, June) and autumn (A, November). In the laboratory, collected samples were thoroughly mixed and homogenized by sieving through a 2 mm sieve. Room-temperature, dried soil was used in chemical analysis. Enzyme activities assays were done on soil samples stored at -20 °C.



*Figure 1.* Mean monthly temperature and precipitation during 2007-2017 at the Khabr National Park and Ruchun Wildlife Refuge

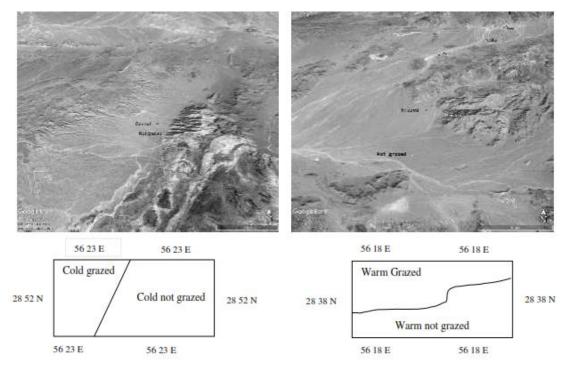


Figure 2. Precise location of grazed and not grazed areas at the cold (left) and the warm (right) sites (Google Earth)

# Soil chemical properties

Soils chemical properties values were measured based on standard methods. The soil pH and electrical conductivity were measured in soil suspensions (Mclean, 1982). Soil water content was measured by drying soil samples at 105 °C (Schlichting and Blume, 1966). The soil texture was determined by the hydrometric method (Toogood, 1958). A wet digestion method (Walkley and Black, 1934) was used to measure soil organic carbon. The soil available phosphorus (AP) was determined using the molybdenum blue method (Olsen and Summers, 1982) following extraction by sodium bicarbonate. A micro Kjeldahl method (Bremner and Mulvaney, 1982) was used to measure total nitrogen (TN) in soil samples.

## Soil enzyme activities

The activity of seven hydrolytic enzymes was determined using their specific substrates. Acid phosphatase, alkaline phosphatase, arylsulfatase and  $\beta$ -glucosidase activity were measured in soil samples based on assays described by Schinner et al. (1996). Invertase and urease activity were determined as described by Schinner and von Mersi (1990) and Kandeler and Gerber (1988), respectively. The enzyme activities were assayed in duplicates at their optimal pH. The controls were soil samples to which substrate were added after the incubation step. An outline of studied enzymes, their substrate and assay pH has been given in *Table 1*.

Enzyme	Substrate	Assay condition (pH)		
Alkaline Phosphatase	p-nitrophenyl phosphate hexahydrate	11		
Acid Phosphatase	p-nitrophenyl phosphate hexahydrate	6.5		
β-glucosidase	p-Nitrophenyl-β-D-glucopyranoside	6		
Invertase	Sucrose	5.5		
Urease	Urea	7		
Aryl Sulfatases	p-Nitrophenyl sulfate	5.8		

Table 1. Soil enzymes assayed in this study with their substrates and assay conditions

# Statistical analysis

The data of variables were checked for their normality with Shapiro-Wilk test. If necessary, the variables were transformed by Jonson transformation using Minitab software to improve normality. Three -way analysis of variance (ANOVA) was used to assessment significant effects of site, season and grazing on soil chemical and enzyme activities. Non-normal data were analyzed using Mann-Whitney U test to evaluate the main effects of studied factors. Mean comparisons were evaluated by the Least Significant Difference (LSD) test at the 5% level of significance. In order to explore links between soil chemicals and enzyme activities, Pearson's correlations were calculated. Reporting of data as histograms was conducted using Microsoft Excel 2010.

## Results

## Soil chemical properties

The soil types are Haplic Calcisoils according to according to the soil classification of the World Reference Base (WRB) for Soil Resources. All soils were characterized as sandy loam texture with an average of 10% clay, 30% silt and 60% sand. None of soil chemical properties was affected by grazing (*Table 2*). Only the effect of season was significant (p < 0.01) on soil pH (*Table 3*). The pH value was higher in the soil samples, collected in autumn (November) than soil samples collected in spring (June) samples (*Table 4*). The effect of site and season was significant (p < 0.01) on total nitrogen (TN) and available P (AP) but not grazing (*Table 2*). Whereas the spring samples had higher available P than autumn samples, the value of TN was more in autumn samples than spring samples (*Table 4*). In addition, site and season interaction was significant (p < 0.01) on AP (*Table 2*) with the highest available AP in samples taken from cold sites in spring (*Fig. 3*).

Turostanonto	Independent variables						
Treatments	EC	TN	AP	SMT			
Site	0.001 <sup>ns</sup>	37.20**	324.00**	13.88**			
Season	0.08 <sup>ns</sup>	6.45**	196.00**	26.62**			
Season × site	0.049 <sup>ns</sup>	0.20 <sup>ns</sup>	$676.00^{**}$	0.03 <sup>ns</sup>			
Replication × (season× site)	1.57	0.49	12.28	0.14			
Grazing	0.03 <sup>ns</sup>	0.04 <sup>ns</sup>	36.00 <sup>ns</sup>	0.03 <sup>ns</sup>			
Season × grazing	0.41 <sup>ns</sup>	0.09 <sup>ns</sup>	36.00 <sup>ns</sup>	0.23 <sup>ns</sup>			
Site × grazing	0.02 <sup>ns</sup>	0.01 <sup>ns</sup>	36.00 <sup>ns</sup>	0.12 <sup>ns</sup>			
Season × site× grazing	0.07 <sup>ns</sup>	0.03 <sup>ns</sup>	36.00 <sup>ns</sup>	0.04 <sup>ns</sup>			
Error	0.001	0.27	12.35	0.15			
Total	53.73	66.07	2030.00	49.36			

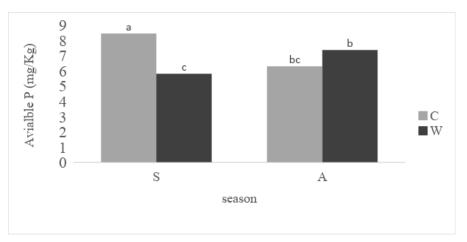
 Table 2. Mean squares of studied factors and their interactions for normalized parameters

Significant effect at p< 0.05 and p < 0.1 levels are indicated by \* and \*\*, respectively. <sup>ns</sup> indicates no significant effect. EC: electrical conductivity, TN= total nitrogen, AP: Available phosphorus and SM: Soil moisture. Transformed data were used in EC and TN analysis.

**Table 3.** Level of significance non-normalized parameters by using non-parametric Mann-Whitney U test

Composition compo	In	Independent variables					
Comparison source	рН	SOC	ACP				
Site (cold and warm)	37.00 <sup>ns</sup>	2.00**	319.00**				
Season (spring and autumn)	$1.00^{**}$	418.00 <sup>ns</sup>	$0.00^{**}$				
Grazing (Grazed with not grazed)	443.00 <sup>ns</sup>	474.50 <sup>ns</sup>	494.00 <sup>ns</sup>				

Significant effect at p < 0.05 and p < 0.1 levels are indicated by \* and \*\*, respectively. <sup>ns</sup> indicates no significant effect SOC: soil organic carbon, ACP= acid phosphatase.



*Figure 3.* Phosphorus availability in soil developed under interaction of site (C= cold and W= warm) and season (S= spring and A= autumn) conditions. Different letters indicate significant difference at the p< 0.05 level

As given in *Table 3*, only site had significant (p < 0.01) effect on soil organic carbon (SOC) with more value in cold sites (*Table 4*). Measured soil water content showed significantly higher values in cold sites and autumn samples in comparison to warm sites and spring samples, respectively (*Tables 2* and 4).

Soil chemical	Si	te	Season		
Son chemicai	Cold	Warm	Spring	Autumn	
pH	7.75 <sup>a</sup>	7.85 <sup>a</sup>	7.9 <sup>b</sup>	8.3 <sup>a</sup>	
Total nitrogen (%)	$0.08^{a}$	$0.05^{b}$	$0.06^{b}$	$0.07^{a}$	
Available phosphorus	35.00 <sup>a</sup>	30.5 <sup>b</sup>	34.50 <sup>a</sup>	31.00 <sup>b</sup>	
Soil organic carbon (%)	$0.65^{a}$	$0.29^{b}$	0.39 <sup>a</sup>	$0.45^{a}$	
Soil moisture (%)	4.44 <sup>a</sup>	1.32 <sup>b</sup>	0.93 <sup>b</sup>	4.83 <sup>a</sup>	

*Table 4.* Mean comparison (n = 8) between sites and season in soil chemical properties

Different letters show significant difference at p< 0.05 level.

### Soil enzyme activities

As shown in *Table 5*, the effect of site (p < 0.01), season (p < 0.01) and grazing (p < 0.05) was significant on alkaline phosphatase activity (*Table 5*). Activity of soil alkaline phosphatase decreased by 28, 22 and 15% in warm sites, autumn samples and not grazed areas in comparison to cold sites, spring samples and grazed areas, respectively (*Table 6*). The effect of site and season was significant on acid phosphatase but not grazing (*Table 3*). Acid phosphatase activity decreased by 32 and 66% in warm site and autumn samples in comparison with cold sites and spring samples (*Table 6*), respectively.

The results indicated significant effect (p< 0.01) of site and season on  $\beta$ -glucosidase and invertase activity (*Table 5*). The  $\beta$ -glucosidase activity decreased by 33 and 52% in warm site and autumn samples (*Table 6*). Grazing did not affect significantly  $\beta$ -glucosidase activity. However, the interaction of site and grazing was significant (p< 0.05) on its activity (*Table 5* and *Fig. 4*).

Treatments	ALP	β-Glu	INV	UR	AS
Site	95256.33**	63936.3**	13.88**	35.57 <sup>ns</sup>	12121.1**
season	40290.02**	$186981.6^{**}$	26.62**	$5353.56^{*}$	7333.90**
Season $\times$ site	4.29 <sup>ns</sup>	6306.15 <sup>ns</sup>	0.03ns	111.22 <sup>ns</sup>	107.53 <sup>ns</sup>
Replication $\times$ (season $\times$ site)	875.566	2127.32	0.14	547.58	128.46
Season × grazing	64.98 <sup>ns</sup>	219.99 <sup>ns</sup>	0.24ns	178.05 <sup>ns</sup>	191.01 <sup>ns</sup>
Grazing	6331.98 <sup>*</sup>	3715.97 <sup>ns</sup>	0.03ns	850.08 <sup>ns</sup>	21.59 <sup>ns</sup>
Site × grazing	503.16 <sup>ns</sup>	$20155.13^{*}$	0.12ns	67.22 <sup>ns</sup>	387.17 <sup>ns</sup>
Season $\times$ site $\times$ grazing	474.31 <sup>ns</sup>	6395.40 <sup>ns</sup>	0.04ns	861.35 <sup>ns</sup>	15.38 <sup>ns</sup>
Error	943.27	316.98	0.15	1000.82	162.56
Total	193852.42	43578.24	49.35	50811.55	28586.39

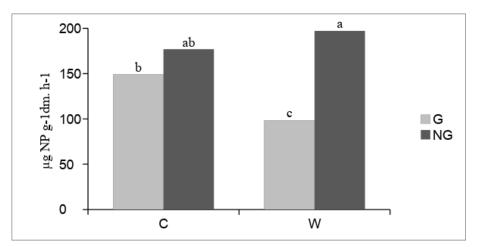
Table 5. A summary of the levels of treatment significance on soil enzyme activities

Significant effects at the p < 0.05 and p < 0.1 levels are indicated by \* and \*\*, respectively. <sup>ns</sup> indicates no significant effect. AlP: alkaline phosphatase,  $\beta$ -Glu:  $\beta$ -glucosidase, INV: invertase, UR: urease and AS: arysulfatase. Transformed data were used in INV analysis.

**Table 6.** Mean comparison (n = 8) between sites, season and grazing on soil enzyme activities

Enzyma	Si	te	Sea	son	Grazing		
Enzyme	Cold	Warm	Spring	Autumn	Grazed	Not grazed	
Alkaline phosphatase	240.68 <sup>a</sup>	163.71 <sup>b</sup>	227.37 <sup>a</sup>	177.19 <sup>b</sup>	162.95 <sup>a</sup>	147.71 <sup>b</sup>	
Acid phosphatase	262.04 <sup>a</sup>	187.89 <sup>b</sup>	374.00 <sup>a</sup>	75.93 <sup>b</sup>	245.12 <sup>a</sup>	238.75 <sup>a</sup>	
β-Glucosidase	186.91 <sup>a</sup>	123.73 <sup>b</sup>	209.38 <sup>a</sup>	101.28 <sup>b</sup>	189.45 <sup>a</sup>	175.12 <sup>a</sup>	
Invertase	2248.39 <sup>a</sup>	528.04 <sup>b</sup>	339.53 <sup>a</sup>	243.69 <sup>b</sup>	1491.45 <sup>a</sup>	1284.7 <sup>a</sup>	
Urease	52.01 <sup>a</sup>	53.99 <sup>a</sup>	61.99 <sup>a</sup>	43.70 <sup>b</sup>	56.49 <sup>a</sup>	49.2 <sup>a</sup>	
Arylsulfatase	120.7 <sup>a</sup>	99.54 <sup>b</sup>	102.60 <sup>b</sup>	124.01 <sup>a</sup>	111.04 <sup>a</sup>	115.4 <sup>a</sup>	

Different letters show significant difference at p < 0.05 level.



**Figure 4.** The significant effect of site (C = cold, W = warm) and grazing (G = grazed, NG = not grazed) interaction on  $\beta$ -glucosidase activity. Different letters show significant difference at P < 0.05 level

There was similar trend in invertase activity, as its activity decreased by 76 and 28% in warm site and autumn samples (*Tables 5* and 6).

Measurement of urease activity showed that only the season caused significant difference on its activity (*Table 5*). The urease activity decreased by 29% in autumn samples in compared with spring samples (*Table 6*).

The effect of site and season was significant (p < 0.01) on arylsulfatase activity (*Table 5*). As shown in *Table 6*, there was a decrease in value of arylsulfatase activity in warm site (17.5%) and spring samples (17%).

#### The correlation between soil properties and enzyme activities

According to Pearson correlation coefficient values, soil electrical conductivity did not show any correlation with other soil chemical properties (*Table 7*). Soil pH correlated negatively with soil available phosphorus and soil moisture (*Table 7*). Whereas the correlation between soil organic carbon and total nitrogen was positive (r= 0.774, p< 0.001), they correlated negatively with soil moisture (*Table 7*).

The Pearson correlation indicated no significant correlation between soil EC and enzyme activities (*Table 7*). Whereas there was no correlation between soil pH and alkaline phosphatase activity, other assayed enzyme activities correlated negatively with soil pH except for arylsulfatase (*Table 7*). The correlation between soil available phosphorous and enzyme activities was positive except for urease and arylsulfatase activity. Total nitrogen showed positive correlation with alkaline phosphatase and arylsulfatase activity (*Table 7*) with higher correlation with arylsulfatase activity (r= 0.609, p< 0.001). The activity of all assayed enzymes correlated positively with SOC contents (*Table 7*). The highest correlation was observed with arylsulfatase activity (r= 0.630, p < 0.001). Based on Pearson values, soil moisture positively correlated with acid phosphatase,  $\beta$ -glucosidase, invertase and urease activity (*Table 7*) except for arylsulfatase activity (r= -0.689. p< 0.001).

### Discussion

In this study, 11 soil variables including soil chemical properties and enzyme activities were measured to determine soil quality in a semiarid area.

Soil pH and EC are known as the principal indicator of the chemical characteristic of a particular soil, playing significant role in soil biogeochemical processes, solubility of soil nutrients, plant growth, microbial growth and enzyme activities (Aciego Pietri and Brookes, 2008; Sinsabaugh et al., 2008). Our results indicated that some of soil chemical properties did not easily change, which supported by other studies (Zarekia et al., 2012; Mureithi et al., 2014; Vargas-Gastelum et al., 2015). The less value of pH in spring samples can be explained by production of  $CO_2$  by plant roots and bacteria that temporarily lower the pH value in natural ecosystem (Subba Rao, 2009).

Available phosphorus (AP), total nitrogen (TN) and soil organic carbon (SOC) are used as important indicators of soil fertility and long-term ecosystem sustainability (da Silva et al., 2008; Wienhold et al., 2009). There are some evidences that plant cover slows losing nutrients from soil (Porder and Chadwick, 2009; Deekor et al., 2012), supporting more TN, AP and SOC content in cold sites with dense plant cover in comparison with warm sites. The less concentrations of SOC at warm sites is attributed to faster turnover of organic C in warm sites and negative correlation between temperature and soil organic carbon (Friedlingstein et al., 2006). Whereas there was no seasonal variation in SOC content, TN and AP changed seasonally. The temporal alteration of available P was contradictory to total nitrogen seasonality. Plants uptake more P with increasing temperature (Yan et al., 2012), depleting the available phosphorus in soil from spring to autumn which is in accordance with our results. The more TN in autumn can be related to more activity of nitrogen fixing bacteria, which possibly increase the TN content (Zeng et al., 2009). In general, seasonal differences in AP and other nutrients are due to change in soil pH, moisture and temperature (Turner et al., 2013).

Variables	EC	pН	TN	AP	SOC	SM	ACP	ALP	βG	IN	UR	AS
EC	1	0.015	-0.096	-0.022	-0.035	0.142	-0.008	-0.053	-0.009	0.065	-0.123	-0.172
pH	0.015	1	0.089	-0.340**	-0.110	-0.567***	-0.835***	0.595	-0.709***	-0.702***	-0.312*	$0.290^{*}$
AP	-0.022	-0.340***	0.078	1	0.206	0.097	0.344**	0.457**	0.414**	0.479***	0.157	0.155
TN	-0.096	0.089	1	0.078	$0.774^{***}$	-0.564***	0.059	0.365**	0.053	0.192	-0.178	0.609***
SOC	-0.035	-0.110	0.774***	0.206	1	-0.508***	0.189*	0.524***	$0.274^{*}$	0.384**	$0.023^{*}$	0.630***
SM	0.142	-0.567***	-0.564***	0.097	-0.508***	1	0.568***	0.040	0.321**	$0.305^{*}$	$0.307^*$	-0.689***
ACP	-0.008	-0.835***	0.059	0.344**	0.189*	0.568***	1	0.681***	0.683***	$0.784^{***}$	$0.308^*$	-0.232
ALP	-0.053	-0.595***	0.365**	0.457**	0.524***	0.040	0.681***	1	$0.578^{***}$	0.706***	0.172	$0.316^{*}$
BG	-0.009	-0.709***	0.053	0.414**	$0.274^{*}$	0.321**	0.683***	$0.578^{***}$	1	0.709***	0.222	-0.068
IN	0.065	-0.702***	0.192	0.479***	0.384**	$0.305^{*}$	0.784***	0.706***	0.709***	1	0.151	0.020
UR	-0.123	-0.312*	-0.178	0.157	0.023	$0.307^{*}$	$0.308^{*}$	0.172	0.222	0.151	1	-0.148
AS	-0.172	$0.290^{*}$	0.609***	0.155	0.630***	-0.689***	-0.232	0.316*	-0.068	0.020	-0.148	1

 Table 7. Correlation values for soil chemical properties and enzyme activities

\*, \*\* and \*\*\* indicate significant correlation at p< 0.05, p< 0.01 and p< 0.001, respectively. EC: electrical conductivity, AP: available phosphorus, TN: total nitrogen, SOC: soil organic carbon, SM: soil moisture, ACP: acid phosphatase, AlP: alkaline phosphatase,  $\beta$ -Glu:  $\beta$ -glucosidase, INV: invertase, UR: urease and AS: arysulfatase.

The soil enzyme activities is the metrics predominantly used to provide information on soil quality. We studied the activity of six extracellular enzymes, involved in P, C, N and S cycling. Soil phosphatases play a major role in the mineralization processes of organic phosphorus substrates. The activity of alkaline phosphatase was more than acid phosphatase, possibly related to soil pH (> 7), which makes condition more favourable for alkaline phosphatase activity than acid phosphatase activity. Whereas phosphatases show pH-dependent activity profile, with specific optimum pH for their maximum activity and stability, only acid phosphatases showed strong negative correlation (r= -0.835, p< 0.001) with soil pH. The soil pH values in studied areas were in the range of near optimum pH for the alkaline phosphatase activity, explaining why this enzyme did not correlate with soil pH (Olander and Vitousek, 2000; Renella et al., 2007).

The positive correlation between alkaline and acid phosphatase activity with SOC and available P is attributed to more activity of these enzymes in cold sites. Generally, a positive correlation is expected between enzyme activities and soil organic carbon, increasing soil enzyme activities as organic carbon increase in soil. The major reason for increased enzyme activities with increasing soil organic carbon could be attributed to the greater availability of soluble organic C, nutrients which stimulate microbial growth and activity as main source of soil enzymes (Debnath et al., 2015). Although

our results showed a positive correlation between available P content and both phosphatase activities, this relationship is usually complicated and not constant, since a positive, a negative or no relationship between them has been reported (Kang et al., 2009; Lemanowicz et al., 2011; Piotrowska-Długosz and Wilczewski, 2014). A significant and positive relationship between phosphatase activity and available phosphorus is observed usually in natural soils and those with a low content of nutrients, where phosphorus deficiency occurs (Šarapatka, 2003). Furthemore, the uptake of available phosphorus by plants may complicate this correlation. The observed positive correlation between total nitrogen and alkaline phosphatase is supported by Piotrowska-Dlugosz and Wilczewski (2014) and Shi et al. (2008) findings. The significant and positive correlation between the acid phosphatase activity and the soil moisture is in agreement with Zheng et al. (2015) and but in contrast with Brockett et al. (2012) findings. The soil moisture content, by altering conditions for soil microbiota, causes changes in soil microbial growth and enzyme activities (Kim et al., 2008; Borowik and Wyszkowska, 2016).

Invertase and  $\beta$ -glucosidase play critical role in C cycle and release low molecular weight sugars that are important as energy sources for microorganisms. The positive correlation between  $\beta$ -glucosidase and invertase activity with soil pH was in consistent with other studies (Piotrowska and Koper, 2010; Shao et al., 2015; Zhang et al., 2015). Although a negative or no correlation between soil pH and invertase and  $\beta$ -glucosidase activity has been reported (Shi et al., 2008; Tan et al., 2014). The positive correlation between  $\beta$ -glucosidase and invertase activity with soil phosphorus is in agreement with Zheng et al. (2015) and Cheng et al. (2013).  $\beta$ -glucosidase and invertase activity were positively correlated with the soil organic carbon content. Similarity, Shi et al. (2008), and Böhme and Böhme (2006) observed significant and positive correlation between βglucosidase and invertase activities with organic carbon content in soil samples, indicating the important role of organic matter in maintaining their activities. In addition, the observed positive correlation between soil organic matter and available phosphorus with invertase and  $\beta$ -glucosidase can explain more activity of these enzymes in cold sites, which is in agreement with various studies (Böhme and Böhme, 2006; Shi et al., 2008; Piotrowska and Koper, 2010). Our results did not confirm any significant correlation between soil total nitrogen and  $\beta$ -glucosidase and invertase, which is in agreement with Shi et al. (2008) and Zheng et al. (2015) but in contrast with Cheng et al. (2013) and Zhang et al. (2015) findings.

Urease is associated with the transformation, biological turnover and bioavailability of nitrogen in soil (Piotrowska-Dhugosz and Wilczewski, 2014). According to our results, there was a correlation between soil urease activity with soil pH (r= -0.312, p< 0.05), soil organic carbon (r= 0.023, p< 0.05), total nitrogen (r= 0.178, p<0.05) and soil moisture (r = 0.307, p < 0.05). Similar results have been reported by Tan et al. (2014), Zhang et al. (2013), and Piotrowska-Dlugosz and Wilczewski (2014). Soil urease activity did not show any correlation with soil EC and available phosphorus, which is in agreement with Melero et al. (2006) and Tan et al. (2014) findings.

Arylsulfatase activity, involved in S cycle, had a significant correlation with soil pH (r=0.290, p<0.05), soil organic carbon (r=0.630, p<0.001), total nitrogen (r=0.609, p<0.001) and soil moisture (r=-0.689, p<0.001) which are supported by Mankolo et al. (2012) and Green et al. (2007). The strong positive correlation between SOC and arylsulfatase highlights the important role of soil organic carbon in arylsulfatase activity (Mankolo et al., 2012).

There is no constant pattern in seasonal variation in soil enzyme activities, depending on assayed enzymes, soil properties and ecosystem types. The enzyme seasonal variation is governed by parameters that regulate enzyme activities such as soil temperature, moisture and substrate availability (Wittmann et al., 2004; Niemi et al., 2005; Baldrian et al., 2008). Although seasonal variations in enzyme activities was not supported by Boerner et al. (2005) and Wallenstein et al. (2008), there was seasonal variation in all studied enzymes. The observed seasonal variation in acid and alkaline phosphatase,  $\beta$ -glucosidase, invertase and urease can be explained by more plant root exudates and seasonal variation in the microbial biomass which is in agreement with Devi and Yadava (2006), Yang et al. (2010) and Wallenstein and Weintraub (2008) findings. The all enzymes had more activity in spring except for arylsulfatase activity. Our finding on more intense activity of arylsulfatase in autumn is opposite with Margesin et al. (2014) report, but can be supported by Whalen and Warman (1996) findings. The more arylsulfatase activity in autumn samples can be related to presence of non-competitive inhibitors, possibly removed by plants during growth season and increasing arylsulfatase activity in autumn samples (Whalen and Warman, 1996).

Although the soil enzyme activities values were more in grazed area but the difference was not significant between grazed and not grazed areas except for alkaline phosphatase activity, showing its more sensitivity to environmental changes. It has been approved that the effect of grazing on soil properties depends on grazing intensity and period (Steffens et al., 2008; Fang et al., 2013).

## Conclusion

It has been accepted that soil chemical properties and enzyme activities are important indicators in soil quality assessment. This study was conducted to illustrate the effect of site, season and grazing on soil chemical properties and enzyme activities. The results revealed the significant influence of site and season on some of soil chemicals and extracellular enzymes activities in semiarid areas but not grazing. The higher activity of all assayed enzyme activity in soils at cold sites can be attributed to the more soil organic carbon as there was a positive correlation between SOC and all assayed enzymes except for urease. There were also significant seasonal dynamics among different enzyme activities. Among the enzymes studied alkaline phosphatase was affected by grazing, which shows its higher sensitivity to soil management. In conclusion, it seems the soil quality is poor in warm sites according to both soil chemical and enzyme activities. Then soil in warm sites are more vulnerable to degradation and need more concern to be protected.

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