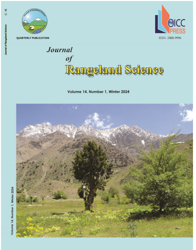


Volume 14, Issue 1, 142404 (1-9)

Journal of Rangeland Science (JRS)

https://dx.doi.org/10.57647/j.jrs.2024.1401.04

Legume-Grass Ratio and some Soil Properties in Four Vegetation Types in Steppe Rangelands of Iran (Case Study: Peshert Rangelands in Chahardangeh Sari, Iran)

Nahid Jabbari[1], Ghasem Ali Dianati Tilaki[1]*, Hasan Ghelichnia[2]

[1]*Department of Range Management, Faculty of Natural Resources, Tarbiat Modares University, Iran.*
[2]*Forest and Rangeland Research Department, Mazandaran Agriculture and Natural Resources Research and Education Center, AREEO, Sari, Iran.*

***Corresponding author:** dianatig@modares.ac.ir

Research and Full Length Article

Received:
30 September 2021
Revised:
10 October 2022
Accepted:
29 October 2022
Published online:
15 January 2024

Abstract:

The Leguminosae family plays a significant role in rangeland ecosystems with biological nitrogen fixation for soil fertility. The grasses significantly increase rangeland production and sustainability. Legume-Grass Ratio (LGR) is an indicator of soil fertility. This study was aimed to investigate the LGR and some soil properties in four vegetation types (*Stipa arabica* Trin.-*Medicago sativa* L., *Festuca ovina* L.-*Astragalus gossypinus* Fisc., *Artemisia sieber* Besser-*Festuca ovina* and *Artemisia sieberi*) in the Peshert rangeland, Chahardangeh Sari, Iran in 2020. In this research, 40 plots of 1 m^2 were randomly established in four vegetation types. In each plot, vegetation characteristics including the number of species per unit area were recorded. Soil sampling was done at a depth of 0 – 20 cm for each plot. Finally, 40 soil samples were analysed in the laboratory for soil properties including: Carbon (C), Soil Organic Matter (SOM), Bulk Density (BD), Aggregate Stability (AS), Particulate Organic Matter (POM), soil texture, available potassium (K), Nitrogen (N), EC and pH, available Phosphorus (P) and carbon to nitrogen ratio. Data were analyzed using one-way ANOVA and Duncan's test was used to compare the differences between four vegetation types for LGR, species diversity and soil properties. SPSS-v20 software was used for data analysis. The results showed that there were significant differences between four vegetation types for all the traits ($P \leq 0.05$). The highest and lowest LGR with average values of 0.86 and 0.10 were observed in *S. arabica-M. sativa* and *Ar. sieberi-F. ovina*, respectively. The lowest amounts of AS%, SOM%, N% and POM% with average values of 0.31, 0.62, 0.03, 3.53 were observed in *Ar. sieberi*. In all vegetation types, soil fertility decreased by decreasing the LGR.

Keywords: Species diversity; Biological form; Soil fertility; Physical properties of soil; Soil nutrients

1. Introduction

Grasses and legumes usually tend to increase soil production and fertility in rangeland ecosystems (Dabighi et al., 2016). Researchers in rangelands have concluded that legumes cause soil fertility by fixing nitrogen biologically (Shafizadeh et al., 2006). In addition to increasing production and rangeland stability, grasses also play an effective role in balancing the ratio of energy to protein and cause the use of nitrogen stabilized by rhizobium strains of legumes (Jafari, 2005). In a study on the effect of *Astragalus cyclophyllon* as a nitrogen stabilizing plant on soil properties, it was concluded that EC and pH of the soil under *As. cyclophyllon* canopy were less than those of the soil without vegetation (Mohammad Ghasemi and Matinkhah, 2017). Legumes improve the soil physico-chemical properties and increase the amount of nitrogen (N) and nutrients in the soil (Dabighi et al., 2016). Suitable forage legumes in the rangeland can increase N and SOM, improve its structure and ventilation and reduce soil erosion and increase soil water holding capacity (Monirifar, 2015).

Figure 1. The location of Peshert rangeland research station in Mazandaran province, Iran

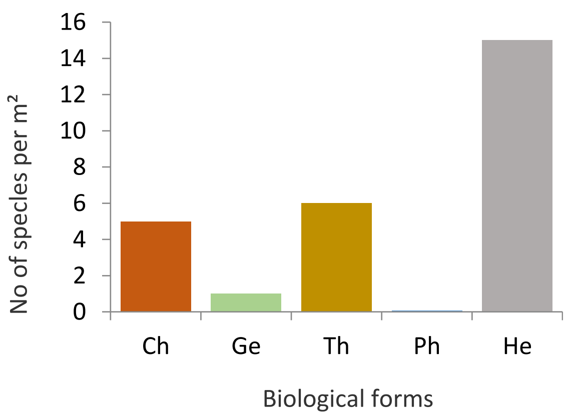
Moreover, legume plants increase the amount of soil nitrogen by stabilizing atmospheric nitrogen (Kouchaki et al., 2015). Annual forage plants of *Leguminosae* family increase soil organic matter and nitrogen while improving soil physical and chemical properties, weed control, biological nitrogen stabilization, and protect soil against erosion and increase soil fertility (Lamei Hervani, 2013; Lauriault and Kirksey, 2004). The percentage of organic carbon in the soil covered with legumes is higher than areas where legumes do not exist. The percentage of soil organic carbon decreases with increasing depth of soil layers (Gharibvand et al., 2013). The use of alfalfa as the best green manure among legume plants significantly increases the soil nitrogen content (Maiksteniene and Arlauskiene, 2004).

Legumes, on the other hand, increase crop production by increasing the amount of nutrients available and the amount of SOM (Belachew and Abera, 2011; Sepehri et al., 2002). Also, legumes with biological nitrogen fixation in rangelands are responsible for the potential contribution to soil fertility, nutrient cycle and crude forage protein increase in rangeland (Muir et al., 2019; Bauters et al., 2016; Mohammadi et al., 2015). Legumes are very resistant to environmental stresses and are of great importance in terms of environmental effects and carbon sequestration (Hassannejad et al., 2013).

The formation of organic matter depends on the amount of N and soil nutrients, which has increased the productivity of other plants due to the entry of N into the soil by legumes (Gill et al., 2002). Legume-grass biomass of 2 tons per hectare controls about 90 % of forages and increases crop production (Bybee-Finley and Ryan, 2018). On the other hand, legume-grass in rangelands improves the growth and yield of associated plants by improving the physical, chemical and biological properties of the soil (Banik et al., 2006). Sepe et al. (2015) in a research on legumes and their role in weed management confirmed that legumes in the rangeland reduce weed growth.

In a study, Nyfeler et al. (2011) showed that the legumes can add more nitrogen to the soil than pure cultivation, which has given considerable functional diversity to rangeland production and agricultural systems. Legume-grass mixture in the rangeland has increased soil moisture, organic matter supply and promoted soil nutrient cycle (Maiti et al., 2021). In the study of legume to grass ratio (LGR) and the role of nitrogen in the improvement of soil nutrients, it was found that the LGR increased nitrogen and soil nutrients (Xu et al., 2020). In a study on woody grasses (Urochloa) and legumes and their role in nitrogen exchange in tropical rangelands of Latin America, Villegas et al. (2020) found that placing legumes next to grasses increased rangeland biomass production by about 74 %, and doubled nitrogen uptake.

The LGR and its effect on the stabilization of N and C stored in soil in temperate steppe rangelands in northeast-



Hemicryptophytes = He, Chamaephytes = Ch,
Therophytes = Th, Geophytes = Ge,
Phanerophyte = Ph

Figure 2. The number of species in a different of biological forms

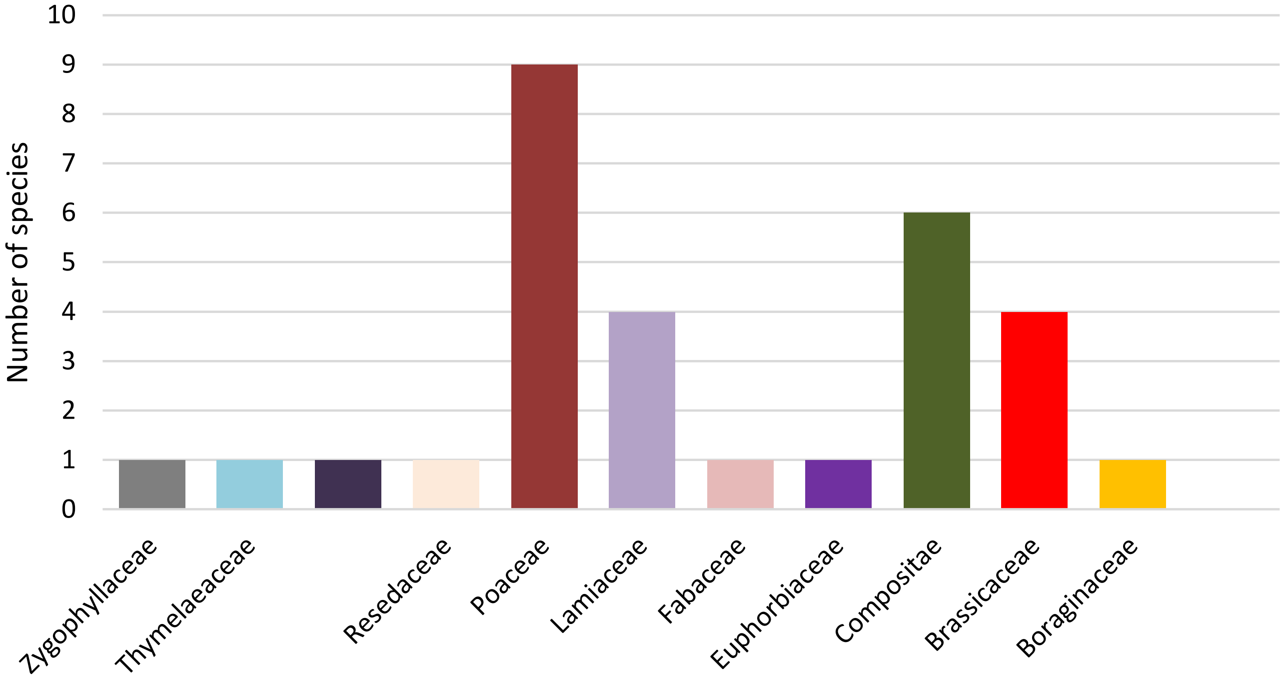


Figure 3. Number of species in each plant family in four vegetation types

ern China showed that legumes increased soil quality and environmental sustainability by increasing soil carbon and nitrogen (Li et al., 2016). LGR is an indicator that can play an important role in proper assessment of rangelands and their management, improvement and rehabilitation (Dianati Tilaki, 2018).

Due to the importance of legumes and grasses in rangelands, the purpose of this study was to investigate the LGR in four vegetation types including: *S. arabica-M. sativa*, *F. ovina-As. gossypinus*, *Ar. sieberi-F. ovina*, *Ar. sieberi* along with changes in soil properties in the steppe region (Fig. 1).

2. Materials and methods

2.1 Study area

In this study, four vegetation types of rangeland plants including (*S. arabica-M. sativa*), (*F. ovina-As. gossypinus*), (*Ar. sieberi-F. ovina*), (*Artemisia sieberi*) were selected. After visiting and identifying the areas, sampling was done randomly. Thus, in each plant type, 10 sample plots of 1 m^2 were randomly selected. Inside each sample plot, rangeland plant species were first identified and then, their numbers were recorded. The Raunkiaer method was used for biological forms classification (Raunkiaer, 1934) and the species diversity was calculated using the Simpson diversity index (Krebs, 1999). Then, soil samples were collected from a depth of 0 – 20 cm. A total of 40 soil samples were taken from four vegetation types and transferred to the laboratory. The soil bulk density was analyzed by the aggregate method (Page, 1982), the soil aggregate stability was measured by the Yoder method (Kemper and Rosenau, 1986; Tajik, 1994), soil texture was determined by hydrometric method, and soil pH was measured using an pH meter in a 1:2.5, soil: water solution. EC (Electrical Conductivity) was determined using an Orion Ionalyzer Model 901 EC meter in a 1:2.5 soil: water solution (Parsapour et al., 2018). Organic C was determined by the Walkley- Black technique (Allison, 1965), and the Micro-Kjeldahl technique was used to determine total N, the soil particulate organic carbon was measured by (Six et al., 1998) method, Available P was determined spectrophotometrically according to the Olsen method (Homer and Pratt, 1961). Available K (by ammonium acetate extraction at pH = 9) was determined with an atomic absorption pectrophotometer (Bower et al., 1952).

In the first step, the normality of the data was examined by Kolmogorov-Smirnov test and the homogeneity of variance of the data was evaluated by Leven test. Pearson correlation between the LGR and soil factors in four vegetation types was determined. One way ANOVA and Duncan's test at 5 % level were used to conduct means comparisons of the LGR, species diversity, soil properties between four vegetation types including: *S. arabica-M. sativa*, *F. ovina-As. gossypinus*, *Ar. sieberi-F. ovina*, *Ar. sieberi*. Statistical analysis of all data was performed using SPSS software version 20.

3. Results

The results showed that there were 27 plant species belonging to 12 families in the area of Poshtkuh Rangeland Research Station (Table 1). The study of biological forms showed that hemicryptophyte, therophyte, geophyte, chamaephyte with 55.55 %, 21.5 %, 4 %, 18 % had the share of flora in the region respectively (Fig. 2).

Fig. 3 showed that the *Poaceae* with 9 plant species had the largest plant family in a total of four vegetation types.

3.1 Vegetation and soil properties in four vegetation types

Result of analysis of variance showed that there were significant differences between four vegetation types for species

Table 1. Scientific name of plant species along with their family and biological forms observed in the geographical region under study area

Scientific name	Families	Biological forms (Rankayer)
Cirsium arvense l.	Asteraceae	Hemicryptophytes
Taraxacum montanum DC.	Asteraceae	Hemicryptophytes
Myosotis arvensis Benefits.	Boraginaceae	Therophytes
Alyssum homalocarpum Boiss	Brassicaceae	Therophytes
Alyssum minus L. Rothm.	Brassicaceae	Therophytes
Artemisia sieberi Besser	Compositae	Hemicryptophytes
Centaurea virgata lam.	Compositae	Hemicryptophytes
Cousinia commutate Bunge.	Compositae	Hemicryptophytes
Euphorbia Aucheri Boiss.	Euphorbiacease	Hemicryptophytes
Astragalus gossypinus Fisch.	Fabaceae	Chamaephytes
Medicago sativa L.	Fabaceae	Hemicryptophytes
Onobrychis cornuta L.	Fabaceae	Hemicryptophytes
Marrubium vulgarewhite Horehound	Lamiaceae	Hemicryptophytes
Teucrium.polium L.	Lamiaceae	Chamaephytes
Ziziphora clinopodioides Boiss.	Lamiaceae	Chamaephytes
Bromus tomentellus Bios.	Poaceae	Therophytes
Cynodon dactylon L. Pers.	Poaceae	Geophytes
Festuca ovina L.	Poaceae	Geophytes(Cryptophytes)
Noaea mucronata Forssk.Aschers. Schweinf	Poaceae	Chamaephytes
Phleum paniculatum Hudson.	Poaceae	Therophytes
Phleum sp.	Poaceae	Therophytes
Poa bulbosa L. var. vivpara Koel	Poaceae	Geophytes(Cryptophytes)
Stipa arabica Trin.	Poaceae	Hemicryptophytes
Reseda lutea L.	Resedaceae	Therophytes
Verbascum gossypinum M.Bieb.	Scrophulariaceae	Hemicryptophytes
Dendrostellera lessertii Wikstr. Van Tigeh.	Thymelaeaceae	Chamaephytes

diversity ($P \leq 0.01$) and LGR ($P \leq 0.05$). The results of means comparison of species diversity between four vegetation types showed that the highest and lowest species diversity amounts with average values of 0.69 and 0.23 were related to (*S. arabica-M. sativa*) and *Ar. sieberi*, respectively (Table 2). The results of LGR in four vegetation types showed that the values of LGR were 0.86 and 0.10 and 0.32 for (*S. arabica-M. sativa*) and *Ar. sieberi-F. ovina* and *Ar. sieberi*, respectively (Table 2).

The analysis of variance of soil properties showed that there were significant differences between vegetation types in sand%, silt%, AS%, bed%, SOM%, total nitrogen%, available potassium%, pH, EC, POM% and carbon to nitrogen ratio ($P \leq 0.05$) (Table 3). For available phosphorus, there was no significant difference between vegetation types. The results of means comparisons of soil properties are shown in Table 3. The results of AS%, SOM%, N%, and POM% in four vegetation types showed that the lowest AS%, SOM%, N%, and POM% with average values of 0.31, 0.62, 0.03 and 3.53 were related to *Ar. sieberi*, respectively. The results of carbon to nitrogen ratio, EC and pH in four vegetation types showed that the highest carbon to nitrogen ratio, EC and pH with average values of 23.85, 1.61 and 8.23 were related to *Ar. sieberi*, respectively (Table 3).

The results of correlation between the LGR and soil factors in four vegetation types showed that there is a negative correlation among LGR with BD of soil ($P < 0.05$). The EC is significantly and negatively correlated with AS ($r = -0.54$; $p < 0.05$). C content is positively and significantly correlated with AS ($p < 0.05$; $r = 0.38$). POM is positively and significantly correlated with AS ($p < 0.05$; $r = 0.50$). N is positively and significantly correlated with AS ($p < 0.05$; $r = 0.42$). C/N is negatively and significantly correlated with AS ($p < 0.05$; $r = -0.35$). P is positively and significantly correlated with AS ($p < 0.05$; $r = 0.38$). Ph is negatively and significantly correlated with

Table 2. Mean (± standard error) of vegetation data in four vegetation types

Factors	Vegetation types				Sig.
	Ar. sieberi	*Ar. sieberi-F. ovina*	*F. ovina-As. gossypinus*	*S. arabica-M. sativa*	
Species diversity	0.23 ± 0.02 b	0.64 ± 0.09 a	0.54 ± 0.23 a	0.69 ± 0.19 a	0.00^{**}
Legume to grass ratio	0.32 ± 0.14 b	0.10 ± 0.10 b	0.83 ± 0.30 a	0.86 ± 0.34 a	0.04^{*}

* and ** = significant at 5 and 1 % probability levels, respectively
Means of rows followed by the same letter (b, a,) has no significant differences using Duncan test ($p < 0.05$).

K ($p < 0.05$; $r = -0.40$). EC is negatively and significantly correlated with K ($p < 0.05$; $r = -0.40$). C is positively and significantly correlated with K ($p < 0.05$; $r = 0.35$). OM is positively and significantly correlated with K ($p < 0.05$; $r = 0.35$). EC is positively and significantly correlated with Ph ($p < 0.05$; $r = 0.33$). C is negatively correlated with EC ($p < 0.05$; $r = -0.54$). OM is negatively correlated with EC ($p < 0.05$; $r = -0.54$). POM is negatively correlated with EC ($p < 0.05$; $r = -0.51$). N is negatively correlated with EC ($p < 0.05$; $r = -0.29$). P is negatively and significantly correlated with EC ($p < 0.05$; $r = -0.34$). OM is positively and significantly correlated with C ($p < 0.05$; $r = 0.33$). POM is positively and significantly correlated with C ($p < 0.05$; $r = 0.31$). POM is positively and significantly correlated with C ($p < 0.05$; $r = 0.31$). C/Nis positively and significantly correlated with C ($p < 0.05$; $r = 0.31$). POM is positively and significantly correlated with OM ($p < 0.05$; $r = 0.33$). C/N is positively and significantly correlated with C ($p < 0.05$; $r = 0.33$). N is positively and significantly correlated with POM ($p < 0.05$; $r = 0.46$). C/N is positively and significantly correlated with N ($p < 0.05$; $r = -0.42$) (Table 4).

4. Discussion

The results showed that the highest LGR with average values of 0.86 was observed in the vegetation type of *S. arabica-M. sativa* as compared to three other vegetation types. One of the reasons for increasing the LGR is the presence of the legume species, which by stabilizing nitrogen leads to increases forage production and soil fertility, which is consistent with the results (Banik et al., 2006; Elijah and Akunda, 2001). The Predominance of the hemicryptophyte biological form in the region is due to its comparative power in the study areas. Safikhani et al. (1999) confirmed our result. The Aggregate stability (AS) was highly dependent on Soil organic matter (SOM) content (Bhattacharyya et al., 2007). SOM increases the AS% by increasing hydrophobicity and adhesion between soil particles (Hosseini and Golchin, 2011). The lowest AS% with average value of 0.31 was observed in the vegetation type of *Ar. sieberi*. The

Table 3. Mean (± standard error) of some physic-chemical properties data of soil in four vegetation types.

Variables	Abbreviation	Vegetation types				Sig.
		Ar. sieberi	*Ar. sieberi-F. ovina*	*F. ovina-As. gossypinus*	*S. arabica-M. sativa*	
Aggregate stability	AS %	0.31 ± 0.03 b	0.40 ± 0.03 a	0.41 ± 0.03 a	0.42 ± 0.02 a	0.002^{**}
Bulk density	BD (g/cm^3)	1.46 ± 0.05 ab	1.35 ± 0.06 b	1.59 ± 0.06 a	1.30 ± 0.08 b	0.016^{**}
Sand	Sand %	44.2 ± 22.66 b	63.0 ± 4.57 a	54.40 ± 3.29 a	35.80 ± 1.62 b	0.000^{**}
Clay	Clay %	29.20 ± 29.20 a	19.80 ± 2.57 b	22.60 ± 1.43 b	33.8 ± 1.99 a	0.000^{**}
Silt	Silt %	26.60 ± 2.02 ab	17.20 ± 2.70 c	23.00 ± 2.24 bc	30.4 ± 1.76 a	0.001^{**}
Soil organic matter	SOM %	0.62 ± 0.12 b	1.31 ± 0.10 a	1.28 ± 0.21 a	1.7 ± 0.12 a	0.006^{**}
Nitrogen	N %	0.03 ± 0.01 b	0.13 ± 0.06 a	0.11 ± 0.007 a	0.9 ± 0.008 a	0.024^{*}
Degree of acidity	pH	8.23 ± 0.04 a	7.94 ± 0.06 b	8.04 ± 0.08 ab	8.16 ± 0.07 a	0.018^{**}
Electrical conductivity	EC (mS/cm)	1.61 ± 1.28 a	1.20 ± 1.06 c	1.24 ± 1.06 b	1.25 ± 0.99 b	0.000^{**}
Available K	K %	2.10 ± 1.39 b	2.93 ± 2.40 a	2.90 ± 2.42 a	1.75 ± 1.60 c	0.000^{**}
particulate organic matter	POM %	3.53 ± 0.30 c	5.54 ± 0.47 a	4.43 ± 0.24 b	5.31 ± 0.26 a	0.000^{**}
Carbon, Nitrogen ratio	C/N	23.85 ± 2.2 a	11.21 ± 1.81 b	7.18 ± 1.19 b	7.63 ± 0.97 b	0.021^{*}
Available P	P %	17.00 ± 7.11 a	24.10 ± 6.36 a	21.00 ± 6.110 a	21.90 ± 9.63 a	0.209^{ns}

ns, * and **, not significant and significant at 5 and 1 % probability levels, respectively
Means of rows followed by the same letter (b, a, c) has no significant differences using Duncan test ($p < 0.05$).

Table 4. Correlation between the legumes-grasses ratio and soil factors in four vegetation types including: *S. arabica-M. sativa*, *F. ovina-As. gossypinus*, *Ar. sieberi-F. ovina*, *Ar. sieberi*.

Traits#	LGR	BD	AS	K	pH	EC	C	OM	POM	N	C/N
BD	-0.34*										
AS	0.13ns	-0.27ns									
K	-0.11ns	0.24ns	0.28								
pH	0.16ns	0.04ns	-0.26	-0.40*							
EC	-0.13ns	0.06ns	-0.54*	-0.40*	0.33*						
C	0.04ns	-0.10ns	0.38*	0.35*	-0.09ns	-0.54*					
OM	0.04ns	-0.10ns	0.38*	0.35*	-0.06ns	-0.54*	0.33*				
POM	0.03ns	-0.26ns	0.50*	0.10ns	-0.06ns	-0.51*	0.31*	0.31*			
N	0.18ns	-0.14ns	0.42*	0.25ns	-0.06ns	-0.29*	0.21ns	0.21	0.46*		
C/N	-0.19ns	0.04ns	-0.35*	0.02ns	0.06ns	0.17ns	0.33*	0.33*	-0.12ns	-0.42*	
P	0.12ns	-0.01ns	0.38*	0.13ns	-0.13ns	-0.34*	0.07ns	0.07ns	0.12ns	0.14ns	-0.01ns

* Significant correlation at 5 % level and ns: non-significant
# = full name of abbreviation listed in Table 3.

increase in soil Bulk density (BD) was due to destructive factors in the region such as erosion. In our study, the lowest percentage of SOM% with average value of 0.62 was related to the vegetation type of *Ar. sieberi*, which can be caused by soil erosion and the transport of organic carbon-rich sediments out of the area through surface runoff, which is consistent with the result of (Al-Rowaily et al., 2015; Olson et al., 2016; Sepe et al., 2015). The results showed that the highest amount of particulate organic matter (POM%) with average value of 3.53 was observed in the vegetation type *Ar. Sieberi*. The increase in the amount of POM% can be due to the volume of roots in the soil. This result is the same as the results reported (Barrios et al., 1996; Handayani et al., 2008).

Our results showed that there was a negative correlation between LGR and soil bulk density. Researchers attributed the lack of nitrogen in the soil to this negative correlation (Ferrero, 1991).

According to the results, the highest amount of N% with average value of 0.9 was observed in the vegetation type of *S. arabica-M. sativa*. Reasons for the increase include plants of the legume family (alfalfa), which have increased soil nitrogen by biological stabilization of N%. This result is similar to those reported (Malakoti and Homaee, 2004; Maiksteniene and Arlauskiene, 2004; Salama, 2020). The results show that the carbon-nitrogen ratio in the vegetation type (*S. arabica-M. sativa*) has the lowest with average value of 7.63 as compared to other vegetation types. The ratio of carbon to nitrogen is an important factor that can affect soil fertility. The lower the carbon to nitrogen ratio, the more suitable the plant is for its soil fertility. The lower the ratio, the lower the resistance of plant residues to degrading agents. That is, they decompose sooner (Saleh Rastin, 1978). The amount of available phosphorus in fine soil particles is more than coarse particles. On the other hand, the average amount of available phosphorus with 24 % in soil type (*Ar. sieberi-F. ovina*) has the highest amount, which can be due to the presence of coarse sand particles with 63 % in this type. This is consistent with the results of (Salar Dini, 2005; Solomon et al., 2002; Malakoti and Homaee, 2004). The results showed that the vegetation types of *Ar. sieberi-F. ovina* and *Ar. sieberi* with average values of 24 % and 17 % had the highest and lowest available potassium levels, respectively. The reason for the high amount of available potassium in the type (*Ar. sieberi-F. ovina*) was due to the high amount of organic matter in this vegetation type, which also increases the amount of available potassium in the soil. The presence of organic matter in the soil causes surface adsorption of these elements and decreases them from eroding. Increasing more OC% under the canopy of plants increases the cation exchange capacity (Gallardo, 2003). The highest soil EC (mS/cm) with average value of 1.61 was observed in *Ar. sieberi*. The results of correlation between the EC and soil factors in four vegetation types showed that there is a negative correlation among EC with AS of soil. According to research results (Kargar et al., 2010), EC is inversely related to the percentage of canopy cover of vegetation and species diversity. Due to the fact that *Ar. sieberi* had the lowest species diversity compared to other types. The lowest pH with average value of 7.94 was observed in the vegetation type (*Ar. sieberi-F. ovina*). The reason for the decrease in acidity can be high amount of organic matter that is produced by decomposition, organic acid and mineral acid, the simplest and most abundant of which is carbonic acid. However, carbon dioxide is a weak acid and its constant production in soils with high density of plant roots causes lime to dissolve and leach from the soil. Extraction of lime from the soil reduces soil acidity, which is a consistent with (Maia et al., 2010) result.

5. Conclusion

GLR in the desired vegetation types (*S. arabica-M. sativa*), (*F. ovina-As. gossypinus*) and (*Ar. sieberi-F. ovina*) improved the growth, and increased hemicryptophytes and the palatable plants by improving the physico-chemical properties of the soil. However, in the *Ar. sieberi* vegetation type, the absence of legumes caused the soil to be less fertile and the palatable plants not to grow properly. In vegetation types such as *Ar. sieberi* and *Ar. sieberi-F. ovina* which had a lower LGR, it had less fertile soil than other types. In all vegetation types, soil fertility decreased by decreasing the LGR. The increase in the density of class I legumes (such as *M. sativa*) decrease the class III grasses and has promoted the LGR which causes the species diversity enrichment in the rangeland.

Ethical approval:
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval does not applicable.

Authors Contributions:
All authors contributed equally to performing experiments, analyzing data, and writing the paper.

Availability of data and materials:
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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