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DOES GRAZING AFFECT NITROGEN COMPOUNDS IN *Neltuma laevigata* IN NORTHERN MEXICO FORESTS?

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Resumo

O pastamento afeta os compostos de nitrogênio em Neltuma laevigata nas florestas do norte do México? As florestas de algaroba *Neltuma laevigata* funcionam como fonte de alimento principalmente para gado de corte. Este estudo foi realizado com o objetivo de verificar se as florestas de algaroba são afetadas pelo pastoreio, principalmente nos componentes de nitrogênio devido à sua importância no crescimento vegetal e no desenvolvimento das árvores. O objetivo deste estudo foi determinar se o pastoreio extensivo influencia a concentração dos componentes nitrogenados, aminoácidos e proteínas em floresta de algaroba. Amostras de raízes e caules de quatro árvores adultas foram coletadas mensalmente em dois povoamentos de algaroba pura, um deles com atividade de pastoreio durante o ciclo produtivo. As concentrações de proteínas solúveis totais (TSP) e aminoácidos totais (AT) foram determinadas por espectrofotometria no UV visível. Os resultados mostraram que o pastoreio tem efeitos de assimilação nos componentes de nitrogênio nas árvores das populações selvagens de algaroba. A raiz e o tronco onde não há atividade de pastoreio apresentam maiores concentrações de componentes nitrogenados em comparação com o local onde o pastoreio é feito como atividade antrópica.
Palavras-chave: Aminoácidos solúveis totais, proteínas totais solúveis, bovinos, vegetação semiárida, fisiologia arbórea.

Abstract

Mesquite forests *Neltuma laevigata* functions as a nourishment source mainly for beef cattle. This study was carried out in order to find if mesquite forests are affected by the grazing, particularly on nitrogen compounds due to its importance in the vegetal growth and tree development. The objective of this study was to determine if the extensive grazing has influence over the concentration of the nitrogen components (total amino acids (TAA) and total soluble proteins (TSP)) in mesquite forest. Root and stem samples in adult trees were taken monthly during twelve months in two pure mesquite stands, one of them with grazing activity. TSP and TAA concentrations were determined by visible UV spectrophotometry. The results shown that grazing has assimilation effects in nitrogen compounds in trees of the mesquite wild populations. The root and stem in the site without grazing activities showed higher concentrations of nitrogen compounds in comparison with the site where grazing is present as an anthropogenic activity.
Keywords: Total soluble amino acids, soluble total proteins, cattle, semi-arid vegetation, tree physiology.

INTRODUCTION

The mesquite (*Neltuma laevigata* [Humb. & Bonpl. ex Willd.] M.C. Johnst.) belongs to the legume’s family. The origins of mesquite species are the American Continent, where a wide genetic variety for different species of the *Neltuma* genre can be found. (ROSALES-SERNA *et al.*, 2011; TRENCHARD *et al.*, 2008). 44 species of the *Neltuma* genre exist worldwide, which 42 species are predominant in the American Continent and only 3 species grow naturally in Asia and one of them is located in Africa (RUIZ-NIETO *et al.*, 2020; ROSALES-SERNA *et al.*, 2011).

In Northern-Central Mexico, the mesquite (*N. laevigata*) was considered one of the plants with greater presence in the past; although, knowing its ecological and economical importance, nowadays the populations have been reduced due to the anthropogenic activities (VALENZUELA-NÚÑEZ *et al.*, 2015).

VALENZUELA-NUÑEZ *et al.* (2015) and TRUCIOS-CACIANO *et al.* (2012) mentions that pressure over the mesquite stands is due to mainly livestock. This has been the main degradation factor in the mesquite ecosystems; it has been observed that an increase in surface of induced grasslands in the areas occupied by mesquite and other legumes.

The perennial nature of the trees depends on the tidy and periodic accumulation of photosynthetic products and related compounds, that accumulate during favorable periods and stock mainly in winter and then move again for the growth and reproduction when the demand arises. They store in the live wood's parenchyma and the cortex in carbohydrates form, lipids and nitrogen compounds (BRICEÑO-CONTRERAS *et al*. 2019; VALENZUELA-NÚÑEZ *et al*. 2010).

The nitrogen compounds, constitute only a small part of the dry weight of the woody plants, but they are very important physiologically. In the live cells, there is a small number of components that contain nitrogen and have important functions in the biochemical and physiological processes.

These components include structural proteins that form the protoplasm and enzymes that catalyze the plant's biochemical processes. There is a big amount of nitrogen in chlorophyl, amides, amino acids, nucleic acids, nucleotides and other nitrogen bases, hormones, vitamins and alkaloids (PALLARDY, 2010). Even though 2% of the plants dry weight is made of elemental nitrogen, in comparison with the 40% carbon, still there is a great number of known organic substances that contain nitrogen in plants.

Nitrogen is an essential micronutrient for the plant's growth. After being absorbed from the soil and assimilated by the plant, the organic ntrogen compounds transport themselves between the organelles, from cell to cell and long distance to support metabolism and plant development (RENTSCH *et al*., 2007). The grazing is the most common anthropogenic perturbation in the grassland's ecosystems (GAO and CARMEL, 2020).

The grazing plays an important part in the biodiversity maintenance (GAO and CARMEL, 2020; YUAN *et al*., 2016) and productivity (GAO and CARMEL, 2020), and also provides humans livestock production by changing the ecological processes. (GAO and CARMEL, 2020; GAO and CARMEL, 2015). Although, in some areas the grazing may cause soil and vegetation degradation (GAO and CARMEL, 2020; PULIDO *et al*., 2016) and reduce even more the livestock production, which has a negative impact. In this study it is intended to determine if the natural mesquite populations are affected by extensive grazing as a food source, mainly for the cattle.

It is necessary to improve more research about the impact of cattle over mesquite populations particularly in nitrogen compounds due to its importance in the vegetal growth and development. Therefore, the objective of this study was to determine if the extensive grazing has influence over the concentration of nitrogen componentes: total amino acids (TAA) and total soluble proteins (TSP) in wild populations of *Neltuma laevigata*. This knowledge is very important in order to develop sustainable animal production, based on a holistic vision and aligned to all productive segments, especially the economic, environmental and sociocultural components; thus generating and promoting strategies aimed to a sustainable use of natural resources (NAVARRETE-MOLINA *et al*., 2019a; 2019b; 2020; RIOS-FLORES *et al*., 2018).

MATERIALS AND METHODS

Study area

This research was performed in two natural stands of *Neltuma laevigata*, the first of them ("Peñoles" without grazing) with an extension of 872 ha and the second one ("El Saladillo", with grazing), with a 978 ha extension in ejido Emiliano Zapata, Cuencamé, Durango. Geographically located at 24°25'53.09" LN y 103°50'41.28" LW and an altitude of de 2,020 m within the physiographical province Mesa del Centro constituted by plains formed by alluvial deposits disrupted by mountains and isolated elevations, mostly volcanic nature. Soils are predominately rendzina and kastanozems. It is found in Hydrological region 36 in the Rio del Peñón sub-watershed where the permanent stream El Saladillo. The climate is mild semi-dry with cool winter and rainy summer. The summer rain is less den 5% BS1kw(w) with an anual mean temperature of 16.5°C and annual precipitation of 526.6 mm (GARCÍA, 1998). The predominant vegetation types are natural grassland and induced grasslands with the main presence of tree species and bushes from the Fabaceae family (INEGI, 2016).

Field sampling

Stem samples were obtained with the help of a chisel, removing the cortex, and for the root a peak tool was used making a small ditch to locate the main root and take out the sample. The samples were carefully cleaned removing soil remains and were placed in perforated foil bags previously labeled; afterwards they were frozen in liquid nitrogen to stop the biochemical processes in the tissues. The samples were sent to the Biology and Ecology Forest Laboratory of Biological Sciences Faculty from Universidad Juárez of Durango State.

Lab work

Samples were stored in an ultra-freezer (Revco Value Plus Thermo Scientific ®) to a -70 °C temperature for a week and afterwards were submitted to a lyophilization process (Labconco Freezone Triad Freeze Dry Systems ®) during seven days for a week in a temperature de -40 °C, to dehydrate the samples and avoid enzymatic activity. The samples were milled in a blade mill (Pulverisette 15 Fritsch®) until a fine dust was obtained.

Determination of total amino-acids concentration

To determine the concentration of the amino acids the Yemm and Cocking methodology was used. Dry matter was weighted by 10 mg in 2 mL micro-tube with an analytical scale (Pioner Ohaus®). 500µL of extraction solution (ethanol/water 70/30) were added to microtubes and leased for 10 minutes. After that, samples were centrifuged (Spectrafuge 16M® Labnet International) to 10,000 rpm at a 4°C temperature for 15 minutes. The extraction of the amino acid solution was placed in a clean 2 mL micro-tube. The extraction was repeated two more times.

The three extractions of amino acids were mixed in one tube, 800µL were taken from the extraction solution and mixed with 200µL of ninhydrin solution. The microtubes were boiled during 5 minutes to 100°C, they were let to cool at room temperature, and put in cells for their lecture in the UV-visible spectrophotometer at 570 nm.

Determination of the total soluble protein concentration

To determine the protein concentration in the samples of *Neltuma laevigata*, 10 mg of dry matter were weighted in an analytical scale and placing them in 2 ml micro tubes, a solution with the protein extraction was prepared 0.1 M (KH_2PO_4, Na_2HPO_4 y PVP 3 %), afterwards an iron pellet was placed into the samples and 1 ml of the extraction solution was added. An agitation in a Vortex ® for 10 minutes was done, so that the cell walls were broken. The samples were centrifuged to 10,000 rpm at 4 °C in a refrigerated microcentrige (Axyspinr Refrigerated Microcentifuge ®) during 15 minutes. After that, 500 µl were extracted from each microtube and placed in spectrophotometer cells, 500 µl Quickstart®, Bradford®, solution was added and agitated gently. Absorbance was taken subsequently to a 595 nm.

Soil analysis

Soil samples were taken at a depth of 30 cm to determine the texture by the Bouyoucos hydrometer method. Quantification of Fe, Cu, Zn and Mn was made by the diethylenetriaminepentacetic acid (DTPA) extracting solution method. Total nitrogen was determined by the Kjendahl method. Determination of organic matter content was made by the Walkley and Black method. Apparent density was determined by the paraffin method. It was determided cation exchange capacity, pH, electrical conductivity. Ca, Mg, and Na were queantified by the paste extract method.

Statistical analysis

Shapiro-Wilk test was applied to prove the normality of the data ensemble. A Levene test was done to prove homoscedasticity. The statistical analysis used for this study was a factorial ANOVA and a test of mean comparison medias (Tukey with a significance level of $p \leq 0.05$) using the statistical software IBM-SSPS 20.0.

RESULTS

Shapiro-Wilk test result for data was abnormal, therefore a logarithmic transformation was made. The results showed a significant difference in the TAA concentration in the root (F=142.66, d. f. = 11, $p < 0.001$), Peñoles site showed the highest concentration in every month in root ($\bar{X}$=3.62 g·100 g^{-1} DM) while Saladillo site showed an mean of ($\bar{X}$=3.03 g·100 g^{-1} DM) in root (Figure 1 and Table 1)

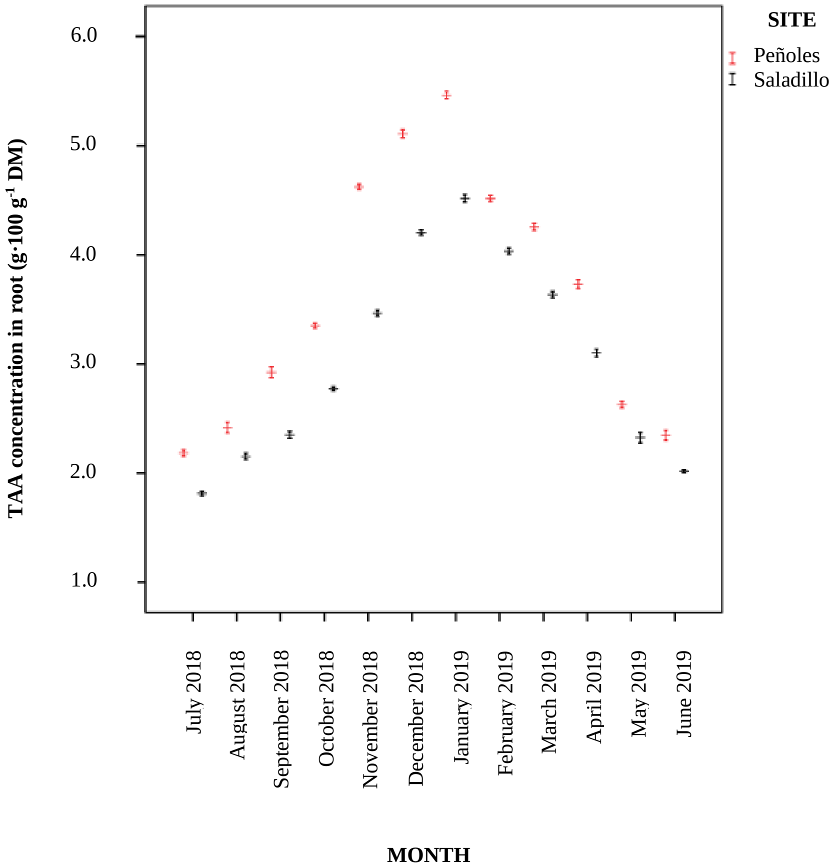

Figure 1. Concentration of TAA (g·100 g^{-1} MS) in mesquite root (*Neltuma laevigata*) in two sites in Durango, México. (Peñoles: without cattle; Saladillo: with cattle)

Figura 1. Concentração de TAA (g·100 g^{-1} MS) em raiz de algaroba (*Neltuma laevigata*) em dois locais em Durango, México. (Peñoles: sem gado; Saladillo: com gado)

Table 1. Inter-subject effect testing of TAA concentration in root and stem of *Neltuma laevigata* in two sites in Durango, México (Peñoles: without cattle; Saladillo: with cattle)

Tabela 1. Teste de efeito intersujeitos da concentração de TAA na raiz e caule de *Neltuma laevigata* em dois locais em Durango, México (Peñoles: sem gado; Saladillo: com gado)

Origin	Square sum Type III	d. f.	Mean square	F	*p*
Root					
Site	3,413.672	1	3,413.672	7,722.697	≤0.001
Month	37,653.595	11	3,423.054	7,743.922	≤0.001
Site * Month	693.693	11	63.063	142.667	≤0.001
Error	159.131	360	0.442		
Stem					
Site	1232.525	1	1232.525	2416.956	≤0.001
Month	25330.485	11	2302.771	4515.685	≤0.001
Site * Month	295.256	11	26.841	52.635	≤0.001
Error	183.582	360	0.510		

a. d. f.= degrees of freedom.
b. F= Tukey test
c. $p \leq 0.05$

A similar effect was observed in the stem (F= 52.63, d. f. = 11, $p < 0.001$), Peñoles site obtained higher TAA concentration ($\bar{X}$=2.60 g·100 g^{-1} DM) in comparison with El Saladillo site ($\bar{X}$=2.24 g·100g^{-1} DM) (Figure 2 and Table 1).

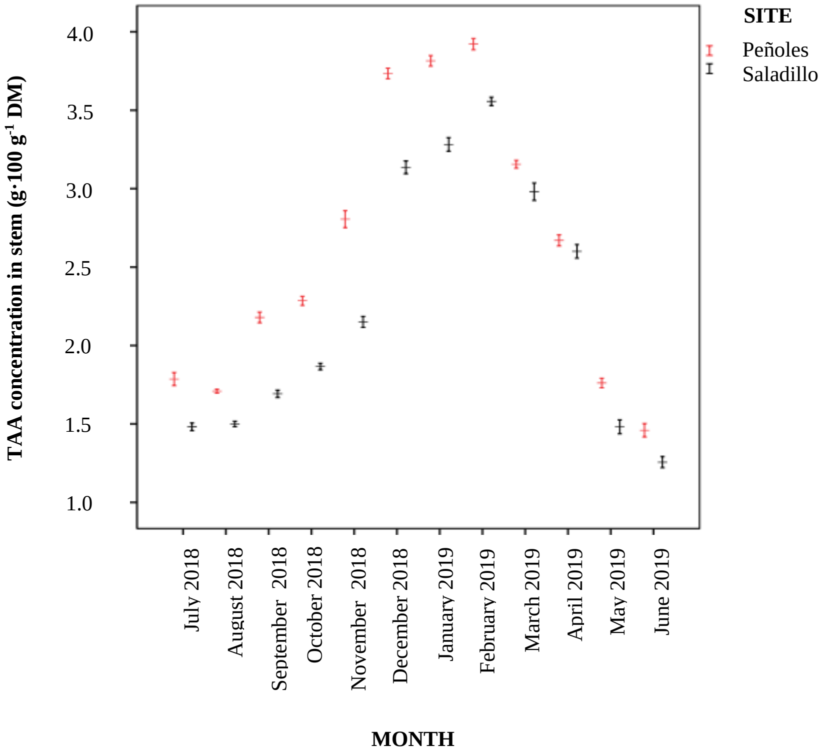

Figure 2. TAA concentration (g·100 g^{-1} DM) in mesquite (*Neltuma laevigata*) stem in two sites in Durango, México. (Peñoles: without cattle; Saladillo: with cattle)
Figura 2. Concentração de TAA (g·100 g^{-1} DM) no caule de algaroba (*Neltuma laevigata*) em dois locais em Durango, México. (Peñoles: sem gado; Saladillo: com gado)

Table 2. Inter-subject effect testing of TSP concentration in root and stem of *Neltuma laevigata* in two sites in Durango, México (Peñoles: without cattle; Saladillo: with cattle)
Tabela 2. Teste de efeito intersujeitos da concentração de TSP na raiz e caule de Neltuma laevigata em dois locais em Durango, México (Peñoles: sem gado; Saladillo: com gado)

Origin	Square sum type III	d. f.	Mean square	F	*p*
Root					
Site	145.911	1	145.911	46.035	≤0.001
Month	2289.137	11	208.103	65.657	≤0.001
Site * Month	375.979	11	34.180	10.784	≤0.001
Error	1141.041	360	3.170		
Stem					
Site	511.185	1	511.185	217.155	≤0.001
Month	1037.140	11	94.285	40.053	≤0.001
Site * Month	381.615	11	34.692	14.738	≤0.001
Error	847.442	360	2.354		

a. d. f.= degrees of freedom.
b. F= Tukey test
c. $p \leq 0.05$

On the other hand, the TSP concentration in the root also shown significant difference (F=10.78, g. l. = 11, p < 0.001), in Peñoles site it was observed higher concentration (x=2.04 g·100 g^{-1} DM) in root, while El Saladillo site showed 1.91 g·100 g^{-1} DM. (Figure 3 and Table 2).

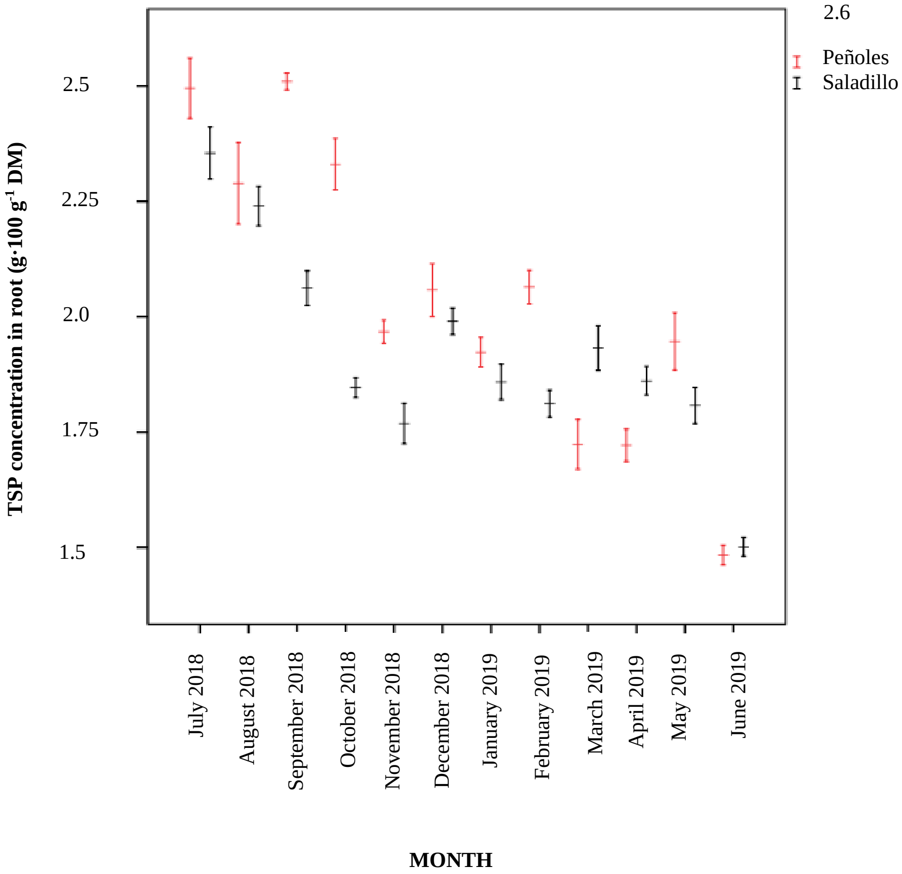

Figure 3. TSP concentration (g·100 g^{-1} DM) in mesquite (*Neltuma laevigata*) root in two sites in Durango, México. (Peñoles: without cattle; Saladillo: with cattle)
Figura 3. Concentração TSP (g·100 g^{-1} MS) em raiz de algaroba (*Neltuma laevigata*) em dois locais em Durango, México. (Peñoles: sem gado; Saladillo: com gado)

The TSP concentration in stem is greater in the Peñoles site, has a mean of ($\bar{X}$=2.10 g·100 g^{-1} DM) opposite to El Saladillo that has a mean of ($\bar{X}$=1.87 g·100 g^{-1} DM), also shown a significant difference in stem between the interactions site: (F=14.73, d. f. = 11, p < 0.001). (Figure 4 and Table 2)

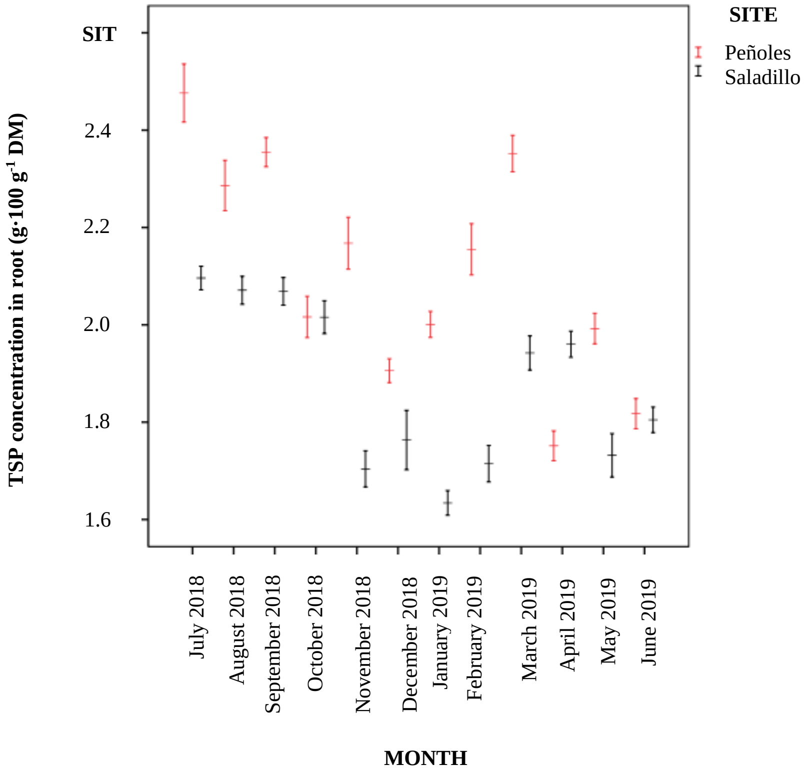

Figure 4. TSP (g·100 g^{-1} DM) in mesquite (*Neltuma laevigata*) stem in two sites in Durango, México. (Peñoles: without cattle; Saladillo: with cattle)

Figura 4. TSP (g·100 g^{-1} MS) em caule de algaroba (*Neltuma laevigata*) em dois locais em Durango, México. (Peñoles: sem gado; Saladillo: com gado)

According to the results, in the case of the TAA concentration in root in El Saladillo site was different (F=3993.47, d. f. 11, $p < 0.001$) with the exception of May and September, the TAA concentration in the rest of the months was different between them, while in September and May were similar among them. In the TAA concentration in root, in Peñoles site a significative difference was observed (F=3911.26, d. f. 11, $p < 0.001$), TAA concentration was different in all months evaluated, except August and June where similarities were observed. Significant difference was found in TAA concentration in stem in El Saladillo site (F=2075.11, d. f. 11, $p < 0.001$), during July, August and May a similarity was found among them, while the rest of the months were different between them. On the other hand, the TAA concentration in stem for Peñoles site also showed a significative difference (F=2501.21, d. f. 11, p <0.001), showing differences in months except August, May, July, December and January.

Differences in TSP concentration in root in El Saladillo were observed (F= 34.96, d. f. 11, p<0.001). In June the lowest TSP concentration was observed ($\bar{X}$= 1.50 g·100 g^{-1} DM), while August and July showed the greatest concentrations ($\bar{X}$= 2.23 and $\bar{X}$= 2.35 g·100 g^{-1} DM, respectively), in the other months similarities were observed in TSP concentrations. In the case of the Peñoles site, TSP concentration in root showed differences (F=40.06, d. f. 11, p<0.001), June showed the lowest concentrations ($\bar{X}$=1.48 g·100 g^{-1} DM) while July and September showed the greatest concentrations ($\bar{X}$= 2.49 y $\bar{X}$= 2.50 g·100 g^{-1} DM, respectively), the root showed similar concentrations rest of the months. A significant difference was observed in TSP in stem in El Saladillo site (F= 22.19, d. f. 11, p<0.001). In this case, January showed the lowest concentrations ($\bar{X}$=1.63 g·100 g^{-1} DM) and the greatest concentrations were observed in July ($\bar{X}$= 2.09 g·100 g^{-1} DM), the rest of the months showed similar results. In TSP concentration in stem for the Peñoles site a significant difference was observed (F=31.28, d. f. 11, p<0.001). It was observed that lower TSP was in April ($\bar{X}$=1.75 g·100 g^{-1} DM) and July showed the highest concentration ($\bar{X}$= 2.47 g·100 g^{-1} DM), while the other months showed a similar behavior in the TSP concentration.

The physicochemical characteristics of the soils in the two study sites are presented in table 3.

Table 3. Soils physicochemical characteristics in the study sites locais em Durango, México. (Peñoles: without cattle; Saladillo: with cattle)
Tabela 3. Características físico-químicas dos solos nos locais de estudo locais em Durango, México. (Peñoles: sem gado; Saladillo: com gado)

Site	CIC	N (%)	pH	EC (m^2 cm^{-1})	OM (%)	Ca	Mg ppm	Na	T
Saladillo	12.5	0.074	6.0	0.350	2.68	2.44	1.54	0.90	Sandy loam
Peñoles	12.0	0.007	6.7	0.485	1.47	1.48	0.56	4.48	Sandy loam

CIC: cation exchange capacity; N: nitrogen percentage; EC: electrical conductivity; OM: organic matter; Ca: calcium; Mg: magnesium; Na: sodium; T: texture

TAA are the main way of nitrogen transportation and trees can translocate up to 50-80% of the nitrogen from the leaf to the stem for its storage during the lethargy stage (BABST and COLEMAN, 2018), this behavior can be observed in figure 4, where TAA concentration reaches its highest value in February.

According to BAZOT *et al.* (2013), the previous studies on deciduous trees demonstrate that nitrogen is transferred from the leaves to the storage organs in the fall. Furthermore, nitrogen mobilizes from leaves in the spring to support the sprout growth. Proteins are the main nitrogen storage way in the vegetative tissues of the trees. Proteins are accumulated at the end of the summer or the beginning of the fall and are found in great quantities throughout the winter (HABERMAN *et al*., 2017), this agrees with the obtained results in this study where it can observed that the greatest TSP concentration was observed in the root during summer in July, August and September. Greatest TSP concentration was maintained elevated during the winter in December, January and February.

The TSP concentration in root remained low during the period of foliar growth in mesquite, these results agrees with BAZOT *et al*. (2013) and DELPIERE *et al.* (2016). In woody plants from mild and cold climate, the vegetative reserve proteins are formed in the roots and stem; its concentration increases in the lethargy stage and decreases in the sprout period (VILLAR *et al*., 2015), this is similar to the results obtained in this study, as it may be observed in the TSP concentration in stem and root.

BYRNES *et al*. (2018) y PULIDO *et al*. (2016) observed that intensive grazing reduces significantly the availability of total nitrogen in comparison to sites where there is no grazing. This is due to the excessive cattle trampling and soil compaction. This leads to decrease soil porosity and that promotes a reduction of infiltration reducing the water available for plants. This explain the diferences observed in both, TSP and TAA concentrations in root in this study, where Peñoles site (without grazing) resulted with greater concentrations in comparison with El Saladillo site (with grazing).

It was observed a low concentration of nitrogen compounds in stem in El Saladillo site (with grazing); this can be due to grazing influence as mentioned by ZUO *et al.* (2018) and DENG *et al*. (2017), who observed that intensive grazing may cause important changes in soil properties, these changes causes coordinated answers to plant's different functional characteristics.

In TPS the Peñoles site (without grazing) greatest concentrations than El Saladillo site (with grazing) were observed, this agrees with the results reported by LIU *et al.* (2018) where they observed that the TSP concentrations in stem and root decreased significantly in an intensive grazing area. BARTHELEMY *et al*. (2017) observed that herbivores also affect the plant's symbiotic interactions, mainly the mycorrhiza's colonization in root. Plants get nutrients from the soil, when the carbon distribution to the roots is reduced due to the loss of photosynthetic tissue caused by the leaves consumed during the grazing.

CONCLUSIONS

- Grazing had effects in the nitrogen assimilation in trees of the wild populations of mesquite. The root and stem in the site without grazing showed higher concentration of nitrogen compounds in comparison with the site with grazing.
- This study was performed during a year. It can be recommended to improve studies during a longer period to determine how grazing affects mesquite wild population in the long term.
- It is also recommended improve this kind of research in younger mesquite individuals in order to know the grazing effects on forest regeneration.
- Management of forests mesquite needs to include a sustainable cattle production, taking into count to the economic, environmental and sociocultural components; thus generating and promoting strategies aimed to a sustainable use of natural resources in mesquite forests.

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