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Seasonal Dynamics of Nitrogen Compounds in a Disturbed Forest in Northern Mexico

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ABSTRACT

Pine and oak forests have great relevance worldwide since they are recognized for the benefits and services they provide. Grazing can affect nitrogen reserves in forest trees in complex ways, the effects manifest through changes in the soil, vegetation, and the nitrogen cycle. Nature tourism can have negative impacts on tree physiology. These impacts affect vital processes such as photosynthesis, respiration, water relations, and metabolism. This study was developed in order to determine whether natural populations of pine (*Pinus cembroides* Zucc.) and oak (*Quercus grisea* Liebm.) are affected physiologically by grazing and tourism during the stages of development and dormancy, specifically in nitrogenous compounds (Total Soluble Proteins [TSP] and Total Amino Acids [TAA]) stocked in perennial organs (stem and roots) and leaves. The results showed that grazing and tourism have both negative and favorable effects on the concentrations of nitrogen compounds. The highest concentration of nitrogenous compounds was found in oak compared to pine. The root, trunk, and leaves in the grazed and non-impacted sites showed the highest concentrations

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of nitrogen compounds compared to the tourism-impacted site. Pine is the species most affected by anthropogenic processes (grazing and tourism) in the stages of development and dormancy, since the concentrations of nitrogenous compounds were lower compared to oak.

Keywords: N Cycle; Fagaceae; Pinaceae; Total Soluble Proteins (TSP); Total Amino Acids (TAA); Grazing; Tourism

1. Introduction

The Mexican pinyon (*Pinus cembroides*) belongs to the Pinaceae family. It grows in temperate climates; it is one of the species with the greatest distribution and great economic and social importance in Mexico; and it is a valuable product in rural communities [1]. The most abundant populations are found in Chihuahua, Durango, Coahuila, Nuevo León, Hidalgo, and Zacatecas [2]. Trees of *P. cembroides* are medium-sized, branched at a low height, and present slow growth; their wood is mainly used as fuel. They grow on dry soils and rocky slopes and can reach up to 15 m in height and a trunk diameter from 30 to 70 cm [3]. The *Quercus* genus has the widest distribution throughout the world [4]. *Quercus grisea* belongs to the Fagaceae family; it grows in the mountainous areas of Mexico, and therefore, the rural communities utilize this type of vegetation to provide ecotourism services [5]. *Quercus* species are widely distributed in the Northern hemisphere, mainly in temperate forests and some in tropical and subtropical regions. The genus includes about 500 species, and it is distributed in the Central, Southern, and Sierra Madre Oriental regions, where the genus reaches its highest representation with around 160 species [6]. The largest distribution of *Quercus grisea* in Mexico is located in Aguascalientes, Chihuahua, Coahuila, Durango, Guanajuato, Jalisco, Nuevo León, San Luis Potosí, Sonora, Veracruz, and Zacatecas [7].

Vegetative reserves are resources accumulated in plants during the dormant period that can be mobilized later for biosynthesis necessary for growth and other functions in the plant during the growing season [8–11]. The vegetative reserves are carbohydrates, lipids, and nitrogenous compounds [12], and they accumulate in the root, stem, and higher organs during periods of abundance (before leaves fall) so that they are available during unfavorable periods (such as winter) in order to support respiration of aboveground biomass [8,9,13,14]. Vegetative reserves not only provide a significant amount of nutrients for future needs, but they can also protect plants by recovering nutrients lost due to tissue aging. Redistribution of nutrients from mature organs to growing active tissues is a highly important strategy for the effective use of potentially limited resources [14,15].

Currently, there are several important gaps and challenges in knowledge about the assimilation and use of nitrogen reserves in forest trees. Most of the studies carried out have focused on a limited number of species. This has caused gaps in knowledge about physiological processes, taking into account that the behavior of nitrogen reserves varies between different tree species and in ecosystems, especially in those that have been disturbed by anthropogenic activities [16,17].

The assimilation of nitrogen reserves in forest trees is a complex and vital process. However, there are many unresolved problems that are of interest to forestry research, focusing on the detailed understanding of genetic mechanisms, the response to changing environmental conditions, and the optimization of nitrogen use efficiency in complex ecosystems. The combined effect of higher temperatures, reduced rainfall, and anthropogenic effects on nitrogen assimilation, especially in the early life stages of trees, is a critical area of research. How these stressors interact to affect nitrogen uptake and metabolism remains unclear. Understanding reserve assimilation at the physiological level is crucial for improving forest management and forest response to climate change [18].

The influence of environmental stress, including drought, high temperatures, and pollution, on nitrogen assimilation and remobilization in trees has not been fully understood. The response of nitrogen reserve dynamics to forest management practices (logging, burning, etc.) varies greatly. It has been observed that some practices can

increase nitrogen reserves, while others can cause losses, depending on the type of vegetation and soil. Therefore, research is needed to determine the best practices for preserving and enhancing nitrogen reserves in forest biomass.

The atmosphere has 78% N_2; however, it is not available for most organisms and can only be used by N-fixing bacteria, which are associated with different vegetable species (such as leguminous), in order to carry out this relevant natural process (passage from the gas form to the one available for organisms) [19]. Total Soluble Proteins (TSP) and Total Amino Acids (TAA) are part of the nitrogenous compounds and are part of the vegetative reserves [13,20]. TSPs have different enzymatic, structural, and functional functions (photosynthesis, biosynthesis, transport, immunity, etc.). The main function of TSP is the formation of new tissues and storing nitrogen as a reserve source [20,21]. The function of TAA reserves is to influence transport and mobilization within the plant [22]. They help tolerate stress when adversities occur, which will lead to avoiding protein synthesis to generate unnecessary energy expenditure [23].

Free-grazing livestock interact with pastoral ecosystems primarily by consuming vegetation, trampling, and spreading excrement [24,25]. Overgrazing becomes a problem when there is an imbalance in natural resources, mainly due to the overload of cattle; however, with adequate management, it has great silvicultural and agroforestry conservation potential, and vegetation can benefit [26]. It has been demonstrated that proper management, such as an adequate animal distribution, vegetal species can counteract the effects of anthropogenic activities [27]. Grazing can affect nitrogen reserves in forest trees in complex ways, with outcomes dependent on the intensity, duration, and type of grazing. Generally, the effects manifest through changes in the soil, vegetation, and the nitrogen cycle [28].

Animals consume vegetation, absorbing nitrogen and concentrating it in their feces and urine, creating zones of higher and lower nitrogen concentrations in the soil. This alters the balance of the nitrogen cycle in the ecosystem. Grazing can decrease total tree biomass, as observed in *Prosopis*, where grazing did not affect protein but did decrease amino acids in the foliage [29].

Conventional tourism is not planned to improve conservation, does not benefit the local rural communities (poor distribution of profits), and can quickly damage a fragile environment; as a result, it can destroy or alter the resources on which it depends [30,31]. The most evident negative environmental impacts of tourist activity are the different types of pollution (water, air, sound, and visual), overpopulation, land use problems, disruption of ecological balance, damage to nature, pollution, and inefficiency in waste management [32]. Nature tourism may have negative impacts on tree physiological processes. These impacts affect vital processes such as photosynthesis, respiration, water relations, and metabolism, compromising tree health, growth, and survival [33]. These negative effects include physical stress from trampling and soil erosion, which can affect the roots' ability to absorb nutrients and thus the formation of nitrogen reserves, as well as soil and water pollution from waste and emissions [31]. Frequent traffic by people and vehicles can compact the soil, damage surface roots, erode the soil, and hinder water and nutrient absorption [31]. The generation of solid and liquid waste, as well as vehicle emissions, can contaminate the soil and water, affecting tree health [34]. Direct contact, such as harvesting branches or using bark for firewood or crafts, can cause physical stress in trees. Light pollution can alter natural cycles and affect the physiology of species that are sensitive to changes in light [34].

It is necessary to improve research in tree physiological processes related to the impact of tourism and grazing on natural vegetation. Knowledge about tree reserves accumulation is very important in order to develop sustainable cattle and tourism activities, based on a holistic vision and aligned to all productive segments, especially the economic, environmental and sociocultural components to generate and promote strategies in order to perform sustainable use of natural resources in northern Mexico forests, particularly in nitrogen compounds (TSP and TAA) due to its importance in the vegetal growth and development. Thus, the main objective of this research was to evaluate the effect of grazing and tourism on the physiology of two forest species, particularly their impact on nitrogen compounds concentrations in a pine and oak forest.

2. Materials and Methods

2.1. Study Area

The study was carried out in the Ignacio López Rayón ejido in Durango, Mexico, in three sites: grazing impact (GI) (24°143'71.6'' N) (−103°763'71.6'' W), tourism impact (TI) (24°165'20.5'' N) (−103°761'66.3'' W) and non-impact (NI) (24°164'99.1'' N) (−103°762'01.5'' W). Sampling was carried out on two dates: summer (September 2022) and winter (January 2023). Samples were obtained from three adult Mexican pinyon trees (*Pinus cembroides*) and three adult grey oak trees (*Quercus grisea*) with an approximate age of 50 years (the age was determined by removing a core with a Pressler drill and counting the growth rings), in an area of 5 ha for each site at an altitude of 2321 m, above sea level. Tree selection was completely random.

2.2. Sample Collection

Two root samples, two trunk samples, and two leaf samples were taken from each tree. The samples were obtained from the root with a conventional pick, digging a small trench to locate the main root, and extracting the sample. Trunk samples were obtained at approximately a height of 1.3 m, removing the bark and obtaining wood samples by a corer (approximately 30 g). Random leaf samples were obtained from different tree branches. The samples were carefully cleaned, removing traces of unwanted material, and placed in previously labelled kraft paper bags to be frozen in liquid nitrogen to stop biochemical processes in the tissues. The samples were transferred on ice at 4 °C to the Forest Ecology Laboratory of the Faculty of Biological Sciences of the Juárez University of the State of Durango.

2.3. Sample Preparation

The samples were placed in a deep freezer (Revco Value Plus Thermo Scientific®) at −70 °C for one week. After that, they were subjected to a freeze-drying process (Labconco Freezone Triad Freeze Dry Systems®) for seven days at −40 °C, in order to dehydrate the samples and avoid enzymatic activity. The samples were minced in a knife mill (Pulverisette 15 Fritsch®) to obtain a fine powder. 10 mg of dry matter (DM) was weighed in microtubes (MCT-200-C of 2.0 mL Clear Axygen Scientific®) in an analytical balance (Adam®PW 250 MAX 250 g d = 0.0001 g).

2.4. Determination of TSP Concentration

To determine TSP concentration, the Bradford method [35] was used. 10 mg of dry matter was weighed on an analytical balance in 2 ml microtubes, and a 0.1 M protein extraction solution (KH_2PO_4, Na_2HPO_4, and 3% PVP) was prepared. A steel ball was placed in the microtubes, and 1 ml of the extraction solution was added. Agitation was carried out in a Vortex® for 10 min to break the cell walls. Samples were centrifuged at 10,000 rpm at 4 °C in a refrigerated centrifuge (Axyspinr Refrigerated Microcentrifuge®) for 15 min, then 500 µl was extracted from each microtube and placed in cells for reading in a spectrophotometer, and 500 µl of solution Quickstart®, Bradford® was added. Cells were shaken and left for 5 min at ambient temperature. Absorbance was read at a wavelength of 595 nm using Bovine Serum Albumin (BSA) as a standard.

2.5. Determination of TAA Concentration

The methodology of Yemm and Cocking [36] was used to determine the TAA concentration. 10 mg of dry matter were weighed in a 2 mL micro tube on an analytical balance (Pioner Ohaus®). 500 µL of an extraction solution (ethanol/water 70/30) was added. The samples were centrifuged (Spectrafuge 16M® Labnet International) at 10,000 rpm at 4 °C for 15 min. The amino acid solution was placed in a clean 2 mL microtube. The extraction was repeated twice. The three amino acid extractions were mixed in a single tube. 800 µL of the extract was mixed with 200 µL of ninhydrin solution in ethanol (2%). The microtubes were boiled for 5 minutes. After that, micro tubes were cooled to room temperature and transferred to cells for reading in the UV-Visible spectrophotometer at 570 nm using leucine as a standard.

2.6. Statistical Analysis

A Shapiro-Wilk normality test was applied. The statistical analysis applied for this study was a two-way

factorial ANOVA, and a Tukey mean comparison test was performed with a significance level of ($p \leq 0.05$) using the IBM-SSPS 20.0 statistical programme.

3. Results

3.1. Concentration of TSP and TAA in Root, Trunk, and Leaves in Development and Dormancy Stages in *Pinus cembroides* and *Quercus grisea* in the Non-Impact Site (NI)

The results obtained in the non-impact site showed no differences between phenological stages in the concentration of TSP in any of the organs of *Pinus cembroides* (**Figure 1**). The results in the non-impact site do not show differences in the concentration of TAA between phenological stages in the root and the trunk (**Figure 1**). In leaves, differences in TAA concentration were observed between phenological stages. In the development stage, the highest value was observed ($\bar{x}$ = 30.50.24 ± 1.18 mg g^{-1} DM) while in dormancy, the lowest value was presented ($\bar{x}$ = 21.03 ± 2.23 mg g^{-1} DM) (**Figure 1**).

In the non-impact site (NI), no differences were observed between phenological stages in the concentration of TSP in the root of *Quercus grisea* (**Figure 1**). In the trunk, differences were observed in the concentration of TSP between phenological stages. In the dormancy stage, the highest value was presented ($\bar{x}$ = 52.68 ± 2.35 mg g^{-1} DM) while in the development stage, the lowest value was observed ($\bar{x}$ = 45.41 ± 1.00 mg g^{-1} DM) (**Figure 1**). Similarly, in leaves, differences were observed in the concentration of TSP between phenological stages. In the dormancy stage, the highest value was observed ($\bar{x}$ = 122.95 ± 4.81 mg g^{-1} DM), while in the development stage, the lowest value was observed ($\bar{x}$ = 46.85 ± 2.64 mg g^{-1} DM) (**Figure 1**).

No differences were observed between phenological stages in the concentration of TAA in any organs in *Quercus grisea* (**Figure 1**).

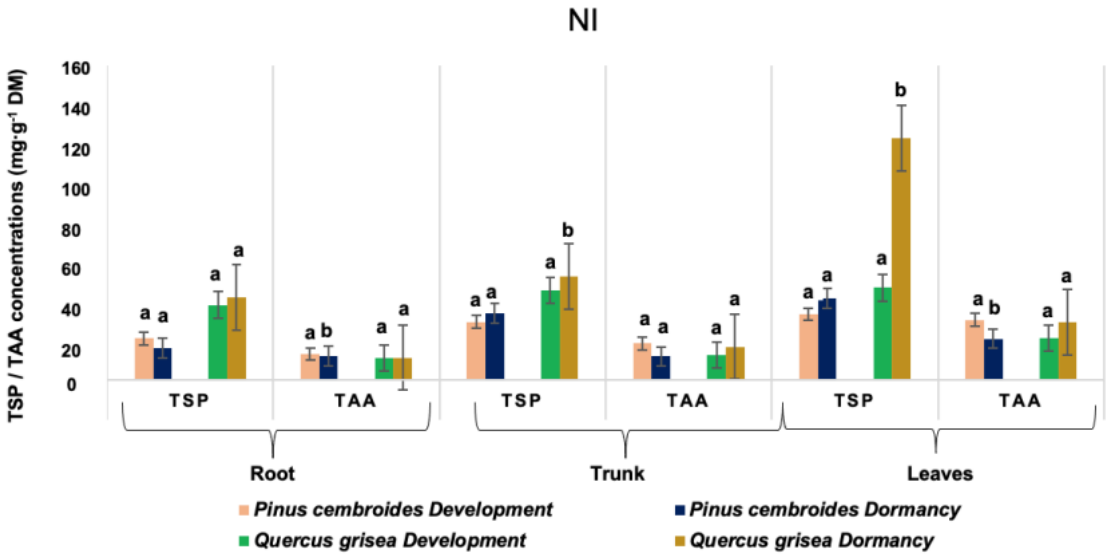

Figure 1. Concentration of TSP and TAA (mg g−1 DM) in root, trunk, and leaves in development and dormancy stages in *Pinus cembroides* and *Quercus grisea* in the non-impact site (NI).

Note: Means with different superscripts (a,b) differ in TSP and TAA concentrations between organs ($p < 0.05$). Vertical bars represent the standard error. n = 3. TSP: Total soluble proteins, TAA: Total amino acids, DM: Dry matter.

3.2. Concentration of TSP and TAA in Root, Trunk, and Leaves of *Pinus cembroides* and *Quercus grisea* in Development and Dormancy Stages in the Site Impacted by Grazing (GI)

In the site impacted by grazing, no differences were observed between phenological stages in the concentration of TSP in any organ in *Pinus cembroides* (**Figure 2**).

No significant statistical differences were observed between phenological stages in the concentration of TAA in the root of *Pinus cembroides* (**Figure 2**). Nevertheless, in the trunk, differences were observed in the concentra-

tion of TAA between phenological stages. In the dormancy stage, the highest value was shown ($\bar{x}$ = 19.30 ± 0.60 mg g^{-1} DM), while in the development stage, the lowest value was presented ($\bar{x}$ = 13.22 ± 1.63 mg g^{-1} DM). In the same way, in leaves differences were observed in the concentration of TAA between phenological stages: In dormancy, the highest value was presented ($\bar{x}$ = 23.52 ± 1.61 mg g^{-1} DM) while in the development stage, the lowest value was presented ($\bar{x}$ = 16.67 ± 1.63 mg g^{-1} DM) (**Figure 2**).

Differences in TSP concentration were observed between phenological stages in *Quercus grisea*. The root in the dormancy stage presented the highest value ($\bar{x}$ = 37.29 ± 0.33 mg g^{-1} DM), while in development, the lowest value was presented ($\bar{x}$ = 29.49 ± 2.87 mg g^{-1} DM) (**Figure 2**). In the trunk, differences in TSP concentration were similarly observed between phenological stages. In the dormancy stage, the highest value was observed in the trunk ($\bar{x}$ = 50.86 ± 1.97 mg g^{-1} DM), while in the development stage, the lowest value was presented ($\bar{x}$ = 33.67 ± 2.26 mg g^{-1} DM) (**Figure 2**). In leaves, differences in TSP concentration were observed between phenological stages: in dormancy, the highest value was observed ($\bar{x}$ = 107.60 ± 2.72 mg g^{-1} DM), while in development, the lowest value was observed ($\bar{x}$ = 61.23 ± 3.62 mg g^{-1} DM) (**Figure 2**).

No differences were observed between phenological stages in the concentration of TAA in the root and leaves (**Figure 2**). In the trunk, differences were observed in the concentration of TAA between phenological stages; the highest value was observed ($\bar{x}$ = 14.69 ± 0.26 mg g^{-1} DM), and in the development stage, the lowest value was shown ($\bar{x}$ = 12.18 ± 0.62 mg g^{-1} DM) (**Figure 2**).

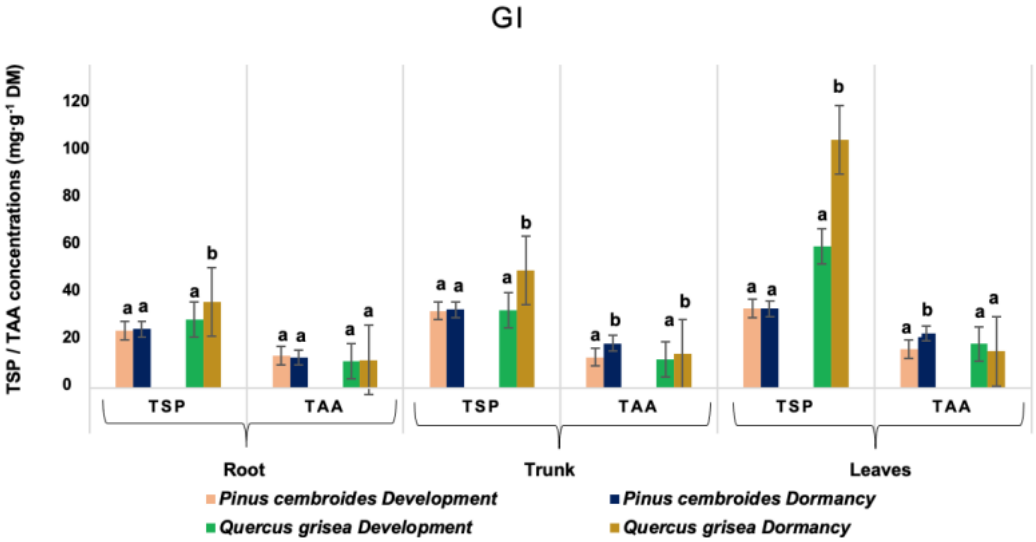

Figure 2. Concentration of TSP and TAA (mg g−1 DM) in root, trunk, and leaves in development and dormancy stages in *Pinus cembroides* and *Quercus grisea* in the grazing impact site (GI).

Note: Means with different superscripts (a,b) differ in TSP and TAA concentrations between organs ($p < 0.05$). Vertical bars represent the standard error. n = 3. TSP: Total soluble proteins, TAA: Total amino acids, DM: Dry matter.

3.3. Concentration of TSP and TAA in Root, Trunk, and Leaves of *Pinus cembroides* and *Quercus grisea* in Development and Dormancy Stages in the Site with Tourism Impact (TI)

In the case of *P. cembroides*, in the root in the site impacted by tourism (TI), differences in TSP concentration were observed between phenological stages. In dormancy, the highest value was presented ($\bar{x}$ = 32.65 ± 2.89 mg g^{-1} DM), while in the development stage, the lowest value was observed ($\bar{x}$ = 13.23 ± 2.11 mg g^{-1} DM). Similarly, in the trunk, differences in TSP concentration were observed between phenological stages, the highest value was observed ($\bar{x}$ = 44.87 ± 9.90 mg g^{-1} DM), while in development the lowest value was presented ($\bar{x}$ = 14.48 ± 2.38 mg g^{-1} DM) (**Figure 3**).

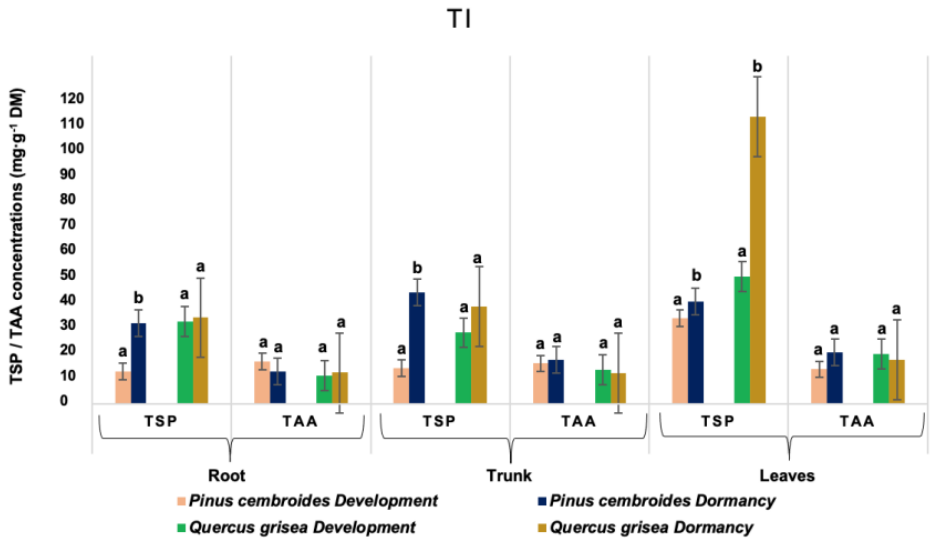

Figure 3. Concentration of TSP and TAA (mg g−1 DM) in root, trunk, and leaves in development and dormancy stages in *Pinus cembroides* and *Quercus grisea* in the tourism impact site (TI).

Note: Means with different superscripts (a,b) differ in TSP and TAA concentrations between organs ($p < 0.05$). Vertical bars represent the standard error. n = 3. TSP: Total soluble proteins, TAA: Total amino acids, DM: Dry matter.

No differences were observed between phenological stages in the concentration of TAA in the root. In the trunk and leaves, no statistical differences were observed between phenological stages in TAA concentration (**Figure 3**).

In *Q. grisea*, the root and trunk in the site impacted by tourism (TI), no differences were found between phenological stages in the concentration of TSP (**Figure 3**). On the contrary, in the leaves, differences were observed in the concentration of TSP between phenological stages. In the dormancy stage, the highest value was shown ($\bar{x}$ = 115.73 ± 4.12 mg g^{-1} DM), while in development, the lowest value was observed ($\bar{x}$ = 51.24 ± 0.89 mg g^{-1} DM) (**Figure 3**). No statistical differences were observed between phenological stages in the concentration of TAA in any of the organs studied (**Figure 3**).

4. Discussion

Deforestation is one of the most relevant problems in environmental policy in Mexico [37]. In Mexico, the original vegetation has been reduced due to the conversion of forest lands to agricultural lands [38]. Livestock interact with ecosystems mainly by consuming vegetation, trampling, and spreading excrement; unless properly managed, livestock tend to concentrate their waste near water sources, feeding areas, and shade [24,25]. This leads to an over-concentration of nutrients in small areas while leaving the rest of the pasture unaffected. Furthermore, heavy and prolonged trampling, especially by large animals like cattle, compacts the soil. This reduces water infiltration, increases runoff, and can lead to severe soil erosion and degradation. In *Quercus robur* trees during October, there is a greater mobilization of TSP for storage [20], which is similar to our results, where the highest concentration of TSP was found in the root and trunk of pine and oak on a similar date of sampling; however, a low concentration of proteins was found in leaves in the dormancy stage. In autumn, trees' focus shifts from rapid growth to preparing for winter, which includes the breakdown and remobilization of TSP, particularly in the leaves [39]. This is a process of recycling and moving the valuable nutrients from dying parts of the plant to permanent storage organs. This was observed in all sites in this research.

In woody plants from temperate and cold climates, TSPs are concentrated in the roots and trunk; this concentration increases in the dormant stage and decreases in the regrowth period [40]. As deciduous trees prepare for winter, they resorb valuable resources from their leaves before shedding them. A large portion of the nitrogen is extracted and transported to the roots. The nitrogen is converted into proteins and amino acids. These compounds are stored in the roots and stems over the winter [41]. This agrees with

the results obtained in the present work, as can be seen in the concentrations of TSP; the highest concentration was found in the root and trunk, with a lower proportion in leaves, becoming more visible in the dormant stage.

Grazing is a problem when natural resources are over exploited; nevertheless, if a management well done is performed, conservation potential and vegetation can benefit [26], grazing in the area of study is not well managed; feet traffic compacts the soil, reducing the pore space necessary for gas exchange and roots require oxygen for respiration and healthy growth, which is essential for the efficient absorption of nutrients, including nitrogen. Compacted soil offers greater mechanical resistance, physically limiting the penetration and expansion of tree roots. A less developed root system has a reduced capacity to explore the soil and absorb available nitrogen. We observed less concentration in both TSP and TAA in the grazed site. These results agree with a study [42] where they found that grazing affected nitrogen reserves in forest trees through changes in the soil, vegetation, and the nitrogen cycle because soil compaction significantly reduces infiltration and water flow through the soil. Water is the vehicle that transports the assimilable forms of nitrogen, nitrate, and ammonium to the roots. Reduced water mobility results in lower nutrient availability for absorption. Grazing can decrease aboveground biomass and lead to a significant reduction in N stored within plant tissues [28], which confirms our results.

It has been observed that moderate grazing may promote soil N sequestration and increase total N, available N, and microbial biomass, N content in the soil compared to no grazing [28]. This is partly due to the return of N through animal excreta and potential stimulation of microbial activity and plant growth [28]. This can be taken into account in forest management plans to achieve a long-term benefit and thereby reduce the negative impact that livestock currently has on the forest resources of the study area.

Actually, ecotourism has a dual impact on Mexican ecosystems: it can generate positive effects like biodiversity protection and local community empowerment, but it also carries negative effects such as pollution, habitat destruction, and resource strain, particularly when not managed sustainably [32]. The success of ecotourism hinges on responsible practices that balance conservation with community benefits and responsible visitor behavior [30,31]. The impact generated by tourism in natural areas is negative and very diverse. These can be classified as: 1) damage caused by the creation of infrastructure, 2) impacts caused by the activities, such as hiking, safaris, bicycle tours, etc., which vary in intensity depending on the type of activity, and 3) damage caused by a large number of visitors in a certain place at the same time [43]. Larger groups cause greater impacts on the natural environment. This coincides with the results obtained in this research, where we can observe that tourism influences the concentration of TSP, showing the lowest concentrations in the root, trunk, and leaves in both stages of development and dormancy of pine and oak trees in the site impacted by tourism.

On the other hand, the most evident negative environmental impacts of tourist activity are the high concentration of people in a single place, the problems of the use of land, ecological breakdown, damage to nature, and pollution with infrastructure and inefficiency in waste management [32]. Ecotourism can negatively impact tree development through soil compaction, physical damage, and various forms of pollution. These stressors compromise tree vitality by disrupting root function, altering nutrient and water uptake, and causing physical injury. The increased soil density creates a physical barrier that prevents roots from penetrating the soil. This leads to restricted root systems, which limit the tree's access to water and nutrients. As a result, nitrogen reserves decrease [43,44]. This is closely related to this research in the low concentrations of TSP and TAA observed in the area affected by tourism in the stage of development and dormancy in a pine and oak forest due to soil compaction.

Amino acids are the main form of nitrogen transport, and trees can mobilize between 50 and 80% of the nitrogen from the leaf to the trunk for storage during the stage of dormancy [45]. This is a crucial part of a process where trees actively alter their metabolism and biochemistry to survive cold temperatures and freezing. When the temperature drops below freezing, water in the spaces outside of tree cells freezes first. This causes water to move out of the cells via osmosis, leading to potentially fatal cellular dehydration. The higher concentration of amino acids and other solutes creates a higher osmotic potential inside the cells, which helps retain water and prevents dehydration. This can be observed in the results of this study, specifically in the root and

trunk, in which TAA concentration is higher in dormancy.

Tourist activities have extensive diffusion and great demand, especially in protected areas, as an important strategy to take advantage of natural resources, it is supported with the argument of little environmental impact that is generated in the natural environment, it can be seen that the large number of visitors at some times of the year, as well as uncontrolled recreational activities in certain natural environments, has caused great impacts to be generated in the environment [46,47].

The damages caused by tourism are greater than the benefits, since they can generate negative impacts on the environment in different ways and at different scales [48,49], the data found in this research showed that the concentrations of TSP and TAA decreased in the site impacted by tourism, mainly to the damage caused by the infrastructure, the fragmentation by roads, damage to the tree trunks and soil compaction.

During long drought periods, cattle consume the shoots from the branches of larger and older trees [50]. Furthermore, the use of wood for construction and for domestic consumption as firewood causes constant and irreparable degradation of the ecosystem, mainly in the physiology of the tree. The results of this study are similar to those of these authors since the collection of pine and oak wood in this forest is notable, causing irreversible damage that is reflected in the low concentrations of TSP and TAA, coupled with the damage caused by the extensive grazing.

On the other hand, nitrogen can reach ecosystems through water in precipitation, and there is a loss through runoff [51,52]. Nitrogen losses depend on the amount of water runoff. This coincides with the results of the present study, where we can observe that the site without impact presented high TSP concentrations compared to the other sites since it presents traces of runoff, which causes the loss of nitrogenous compounds through soil leaching.

5. Conclusions

The root and trunk were the organs that represented the largest reservoir of reserves during the winter in both species.

The methodology described in this study allowed for a reliable analysis of nitrogen compounds, since sampling involves significant physical effort to obtain samples.

Grazing had negative effects on the concentration of both TPS and TAA; nevertheless, it is possible to get benefits in the forest with moderate grazing.

Tourism has a negative impact on pine and oak forests since the alteration it suffers directly influences the concentrations of nitrogen compounds, thus limiting the development of these species and even causing the fragmentation of these ecosystems.

Further studies can be conducted, taking into account factors such as the number of tourists visiting the area used for tourism. In the case of the grazing area, the number of animals and their species can be considered.

The species most affected by anthropogenic processes in the studied area was *Pinus cembroides*; in the site with impact from tourism, the lowest concentration of nitrogenous compounds in the root and trunk was found in this species.

The present study was developed in adult trees; it is recommended that this study be replicated in young trees, in order to compare TSP and TAA reserves at different ages of these species.

Author Contributions

L.M.V.-N.: writing, statistical analysis, sampling, project design; A.G.-I.: writing, sampling, statistical analysis; V.C.-V.: writing, traduction, statistical analysis; D.I.C.-M.: writing, statistical analysis; E.A.B.-C.: writing, sampling; J.C.R.-S.: writing, sampling; C.N.-M.: writing, sampling. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data are unavailable due to privacy. Contact the corresponding author if interested in collaborative work.

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Conflicts of Interest

The authors declare no conflict of interest.

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