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Diversity Levels under Different Grazing Intensities in Semi-Wet Grasslands

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Abstract: The biodiversity of grasslands has been shaped by long-time interaction between natural processes and human activities, such as grazing. Traditional grazing management by animals contributes to the maintenance of high biodiversity in grasslands. However, changes in land use in recent years such as the abandonment of animal husbandry or intensification of grazing, pose a threat to grasslands biodiversity. The aim of the present study was to investigate the compositional and functional diversity in semi-humid grasslands of northern Greece under different grazing intensities. Three sites with similar vegetation and composition but different grazing intensities were selected in Taxiarchis University Forest of Mountain Holomontas. The three grazing intensities were (1) ungrazed, for more than 40 years, (2) moderate grazed, and (3) highly grazed. The ungrazed area showed an increase in vegetation cover and a decrease in bare ground. In comparison to grazed areas, the ungrazed one showed lower levels of grasses and legumes but higher abundances of woody and broad-leaved species. The highest values of all the studied diversity indices (Shannon–Wiener (H), Simpson Diversity Index, Jaccard, and species abundance) were recorded at moderate grazing intensity. Differences were recorded between the grazed and ungrazed areas in all of the quantitative traits (specific leaf area, leaf dry matter content, stem dry matter content, vegetative and reproductive plant height, leaf nitrogen, and phosphorus concentration). Protecting sensitive mountainous grasslands from both overgrazing and abandonment requires a balanced and sustainable management approach.

Keywords: grassland ecosystems; grazing intensity; diversity; cover; composition; functional traits

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1. Introduction

Grasslands occupy approximately 54% of terrestrial ecosystems [1,2] and are the largest natural ecosystem on earth. Grasslands in Greece represent around 40% of the total surface of the country, a number that defines them as the largest land-use resource [3]. Grasslands play a crucial role in supporting biodiversity. Specifically in Europe, 18.1% of endemic plant species are found in grasslands [4] as well as a large proportion of vertebrate and invertebrate species [5].

The biodiversity of grasslands has been shaped by the long-term impact of human activities, such as grazing [6,7]. Particularly, extensive traditional management has played an important role in the high biodiversity of Mediterranean grasslands [8]. In recent years, however, changes have been observed in the way they are managed, either due to the abandonment of animal husbandry, mainly in the mountainous areas, or due to intensification [9,10]. Those changes pose threats to their biodiversity [4].

Mediterranean grasslands, often developing in shallow soils, are highly vulnerable to both management changes and climate variations. Grazing in these ecosystems affects the structure and function of plant communities, leading to alterations in species composition. As a result, grazing is a critical factor influencing their diversity, which serves as an indicator of their condition and stability.

The initial assumption regarding the impact of grazing intensity on floristic diversity was that species diversity increases under moderate grazing intensity. Later, Huston [11] and Kondoh [12] argued that moderate grazing intensity enhances floristic diversity in high-productivity grasslands, while reducing it in low-productivity ones. According to this hypothesis, Milchunas et al. [13] added the "grazing history" factor. Nevertheless, research on grasslands, regarding the impact of grazing intensity on floristic diversity has yielded conflicting results, such as an increased, decreased, or insufficient response [14,15]. According to the theoretical model proposed by Pulungan et al. [16], floristic diversity increases with moderate grazing intensity when (i) grazing is the primary disturbance factor for the plant community and (ii) species composing the plant community differ in their response to grazing.

Classical assessments of ecosystem floristic diversity were based on species composition and were expressed through various indices. However, plant species within a community differ in morphology, physiology, and reproduction, consequently influencing the ecosystem functions differently. This led to a shift in research towards functional diversity. According to Ma et al. [17], the floristic diversity of plants is defined as the range and dispersion of plant characteristics within a community, landscape, or ecosystem that are functionally related to growth, reproduction, and survival. It is considered a significant component of biodiversity. Recent studies have shown that the functional traits of species in a plant community are more effective tools for predicting the response of the community to disturbances such as grazing, compared to species composition. Plant functional types classify species into groups with similar combinations of functional traits, exhibiting similar reactions or sensitivities to environmental or anthropogenic disturbances [18,19]. For classifying species based on their response to grazing, a group of functional characteristics related to the distribution, establishment, and survival of species in the community has been proposed and used [20]. However, the value of these functional traits as predictive tools for the response of the plant community to grazing in different vegetation types and climatic conditions is still under investigation [21]. The results of this research indicate that, for the same functional traits, species show different responses under different environmental conditions [22]. Based on these data and for the improvement in the approach to functional diversity, Diaz et al. [22] have suggested the use of specialized functional traits for each ecosystem.

The use of functional traits to describe and classify plant species into groups based on their response to grazing, in order to predict the stability of plant communities and the functioning of ecosystems, is constantly increasing. However, research regarding the relationship between species diversity and the diversity exhibited by functional characteristics within species is limited [23].

The aim of the present study was to detect the effect of different grazing intensities on species composition and floristic and functional diversity in mountainous semi-humid grasslands of northern Greece. The research focused mainly on the effect that the abandonment of grazing has on the grassland ecosystem, as a rapid decline in livestock farming has been recorded in mountainous areas in recent decades.

2. Materials and Methods

2.1. Study Area

The research was conducted in Taxiarchis University Forest located on the southern and southwestern slopes of Mountain Cholomontas (40°23′–40°28′ N, 23°28′–23°34′ E), at an altitude of approximately 800 m. The total area of the University Forest is approximately 5.800 ha and it is estimated that approximately 4.620 ha are freely grazed mainly by goats and sheep (Management Plan of the University Forest of Taxiarchis-Vrastama 2012–2021) (Figure 1).

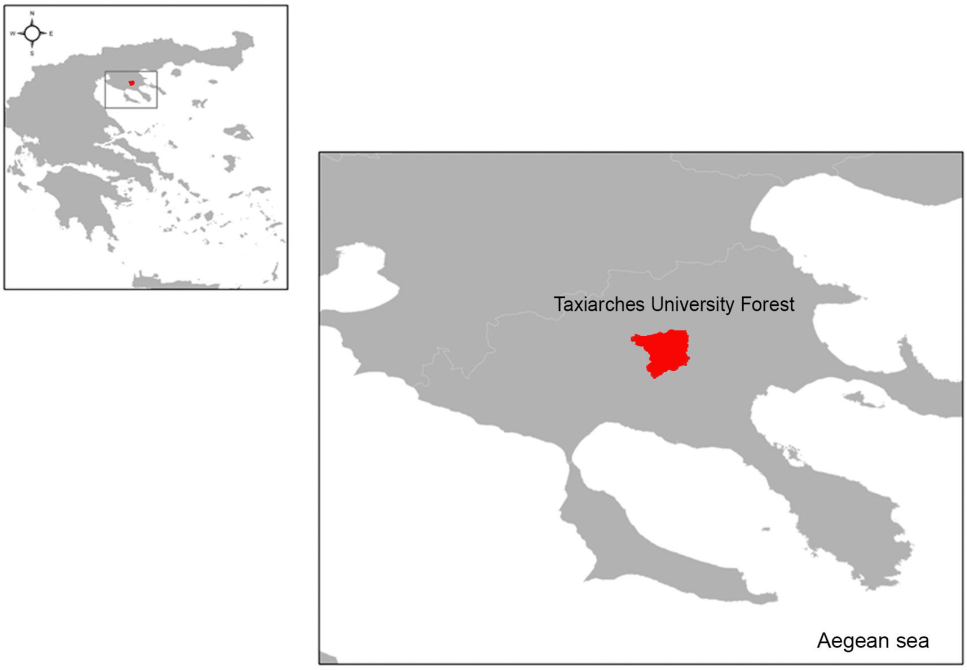

Figure 1. Map of the research area (1:10,000).

The climate of the study area is characterized as terrestrial Mediterranean with short, warm, and dry summers and mild winters. The main feature of the climate is the significant fluctuations in rainfall, with peaks in November–December and May–June. The average air temperature is 11.3 °C, the average maximum air temperature is 15.1 °C, and the average minimum air temperature is 7.1 °C. The coldest months are January and February, with an average temperature of 2.0–2.5 °C, while the warmest months are July–August, with an average temperature of 20.9–21.4 °C.

The average monthly temperatures for each year of measurements (2013, 2014, and 2015) were 12.61 °C, 12.45 °C, and 12.08 °C, respectively, and the precipitation amounts were 749.9 mm, 1320.7 mm, and 822.8 mm, respectively. However, during the months of measurements (May and June), the temperature range and precipitation varied. In 2013, the average monthly temperatures for May and June were 17.64 °C and 19.34 °C; in 2014, they were 14.7 °C and 19.01 °C and in 2015, they were 16.41 °C and 17.72 °C. Similarly, the average precipitation for the year 2013 was 88.6 mm and 65.4 mm for each month, for 2014 it was 52.3 mm and 41.3 mm for each month, and for 2015 it was 48.6 mm and 110 mm for each month. It is observed that while 2014 had the highest total precipitation, during the critical months of May–June (Supplementary Materials), it had the lowest rainfall compared to the other two years. Additionally, relatively low precipitation was recorded in the autumn and winter of 2013 (Supplementary Materials). In contrast, in 2015 (Supplementary Materials), the highest rainfall was recorded in May and June, with high rainfall also during the autumn and winter of 2014 (Supplementary Materials).

Three sites with similar vegetation and composition but different grazing intensities were identified: (1) ungrazed (forage utilization percentage = 0% for more than 40 years), (2) moderate grazed (FUP 30–50%), and (3) highly grazed (FUP > 60%) (Management Plan of the University Forest Taxiarhis–Vrastamon 2012–2021) (Figure 2). The ungrazed area belongs to a private owner and has not been grazed for more than 40 years. Each selected area for all three grazing treatments corresponds to approximately 2 hectares (Figure 2). The FUP was estimated by the Ocular estimate-by-plot method [24]. Forage utilization is defined as the amount or percentage of the current growth of forage that has been removed by grazing animals and can be applied to single plants, plant groups, or the grazing land as a whole [25].

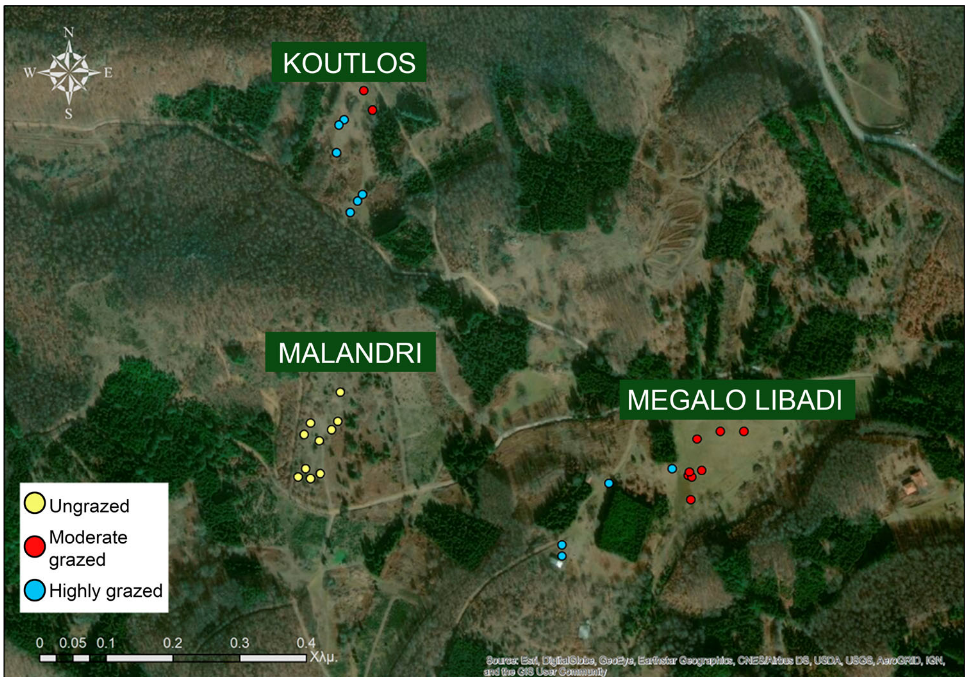

Figure 2. Map of the three grazing intensities. Each dot represents the permanent transect lines for each grazing intensity.

2.2. Vegetation Cover and Composition

In each site, 10 permanent transect lines of 25 m long were taken randomly and measurements of the cover of the vegetation were taken using the line-point method [26]. Species composition was derived from the measurements of cover, after deducting the dry matter, soil, and stones. Plant species that could not be identified in the field were collected and transported to the laboratory. Flora Europaea [27–31] and Flora Hellenica [32,33] were used to classify the collected plant samples into taxonomic units. For the nomenclature of all the taxa, papers of Dimopoulos et al. [34,35] were used as well as the database created from these papers and published on the website https://portal.cybertaxonomy.org/flora-greece/intro (accessed on 29 January 2024). For the determination of species composition, plant species were classified into grasses (Poaceae), legumes (Fabaceae), forbs, which include the herbaceous plants of all the other families, and woody species. Furthermore, the grazing effect on the cover of each dominant species in ungrazed plots (ΔGE dc) was expressed as

$$\Delta\mathrm{GE\ dc} = \frac{(\mathrm{G} - \mathrm{UG})}{(\mathrm{G} + \mathrm{UG})}$$

where G and UG are the coverage percentages of the species in moderate or highly grazed areas and in the ungrazed area, respectively. As the index approaches 1, species are favored by grazing, while the smaller the index is, grazing reduces the presence of the species [36]

Also, the forage value index was calculated according to Klapp-Stahlin [37,38] after being equalized by the percentage of cover of each species. The forage value index assesses the favorability of a plant species while they grow in their natural state within the plant communities.

2.3. Floristic Diversity

The measurements of floristic diversity were made along the transects, which were used to measure cover and species composition based on the line and point method. In each plot, 10 transect lines of 20 m were placed at random locations and then 0.5 × 0.5 m sampling frames were used. Four frames were placed at 5 m, at 10 m, at 15 m, and at 20 m Then, on a special form, the species and the number of individuals of each species were recorded. In case that plant species could not be identified in the field, samples were collected for their identification in the Laboratory as above (Section 2.2).

Moreover, the species number, the diversity indices of Shannon–Wiener, Simpson, evenness, β-diversity, equitability, as well as a Jaccard index, were estimated for each grazing intensity.

The diversity indice of Shannon–Wiener was estimated by the following formula:

$$\mathrm{H} = -\Sigma \mathrm{pi} * \ln \mathrm{pi}$$

where H = diversity indice of Shannon–Wiener, Σpi = the sum of the number of species, and pi = the proportion of individuals of i species in the total sample.

The diversity indice of Simpson was estimated by the following formula:

$$\mathrm{D} = \Sigma\left(\frac{\mathrm{n}}{\mathrm{N}}\right)$$

where D = diversity indice of Simpson, n = the total number of individuals of a particular species, and N = the total number of individuals of all species.

The evenness of plant species (J) was estimated by the following formula:

$$\mathrm{J} = \frac{\mathrm{H}}{\mathrm{Hmax}} = \frac{\mathrm{H}}{\ln \mathrm{N}}$$

where J = eveness, H = the value of diversity index, and Hmax = the maximum possible value of the diversity index (H), which is calculated as the logarithm of the number of species per frame in each transect.

β-diversity was estimated by the following formula:

$$\beta = \frac{\mathrm{ST}}{\mathrm{Smean}}$$

where ST = total number of species for each grazing intensity and Smean = the mean number of species which recorded in each transect for each grazing intensity [36,39].

The Jaccard index was estimated by the following formula:

$$\mathrm{Cj} = \mathrm{j}/(\mathrm{a} + \mathrm{b} - \mathrm{j})$$

where j = the number of species common to both sites, a = the number of species in site A, and b = the number of species in site B.

2.4. Functional Traits of Plant Species

The protocol described by Cornelissen et al. [40] was followed in order to measure the functional traits of species. During the field measurements, plant samples were collected in order to identify and determine species composition in the study area and at the three grazing intensities. The plant samples, after being dried, were identified based on Flora Europea [27–31]. Plant nomenclature was based on the site http://portal.cybertaxonomy.org/flora-greece/intro (accessed on 29 January 2024).

The following plant traits were investigated: specific leaf area (SLA), leaf dry matter content (LDMC), stem dry matter content (StDMC), leaf phosphorus concentration (LPC), leaf nitrogen concentration (LNC), vegetative plant height (VPH), reproductive plant height (RPH), plant defense mechanisms against grazing, life form, life cycle, and photosynthetic pathway.

Specific leaf area is the one-sided area of a fresh leaf divided by its oven-dry mass expressed in m^2kg^{-1} or correspondingly in mm^2mg^{-1} [40]. Leaf dry matter content is the dry-oven mass (mg) divided by its water-saturated fresh mass (g), expressed in mg g^{-1} [40]. Leaf nitrogen and phosphorus concentration are total amounts of N and P, respectively, per unit of dry leaf mass, expressed in mg g^{-1} [40]. Stem dry matter content is the dry-oven mass of a section of a plant's main stem divided by the mass of the same section when still fresh. It is expressed in mg mm^{-1} [40]. The vegetative and reproductive height is the shortest distance by the upper boundary of the main photosynthetic and reproductive tissues on a plant and the ground level, expressed in meters [40].

Specific leaf area (SLA) (Section 3.3.1), leaf dry matter content (LDMC) (Section 3.3.1), stem dry matter content (StDMC) (Section 3.3.1), leaf phosphorus concentration (LPC) (Section 3.3.2), leaf nitrogen concentration (LNC) (Section 3.3.2), vegetative plant height (VPH) (Section 3.3.3), and reproductive plant height (RPH) (Section 3.3.3) are quantitative (continuous) characteristics and were measured on the experimental areas; all the other measurements were carried out in the Laboratory of Grassland Ecology of AUTH. Plant defense mechanisms against the grazing, life form, life cycle, and photosynthetic pathway (Section 3.3.4) are qualitative (categorical) characteristics and were gathered from bibliographic sources and field observations. Data collection was performed based on the species composition for each grazing intensity.

To identify the dominant herbaceous species, those that collectively accounted for 80% of the species composition on each grazing intensity were calculated. According to Garnier et al. [41], this assumption ensures a satisfactory description of ecosystem properties. Also, Grime [42] emphasizes that the functioning of an ecosystem is significantly influenced by the dominant species participating in its composition.

The leaf surface was measured using the software Image-Pro 6.3 for Windows (Figure 3). The specific program scans the area of the leaves and then it automatically calculates the perimeter, area, size, and other parameters. Before carrying out the measurements, the accuracy of the Hewlett–Packard Scanner was checked and verified by using pieces of paper of known area and similar shape and size according to the leaves that were measured.

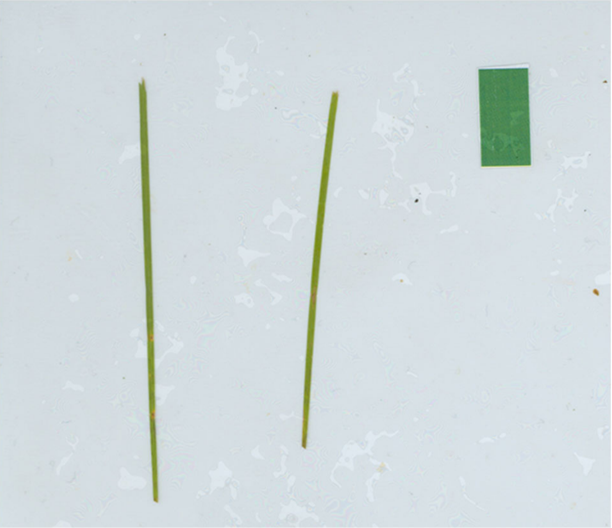

Figure 3. Leaf surface of *A. canina* was measured using the software Image-Pro 6.3 for Windows.

2.5. Statistical Analysis

All data concerning plant cover, species composition, and all floristic diversity indices were analyzed statistically by using a two-way ANOVA in SPSS ver. 21 for Windows package [43] based on the general linear model (general linear models—GLM) with main plot factor grazing intensity and sub-factor the year of measurements. Vegetation transects for coverage and composition measurements were considered replications. If statistically significant differences were found, the averages were checked with the test of least significant difference—LSD (least significant difference) [44]. All comparisons were made at the 5% significance level ($\alpha = 0.05$).

A principal component analysis (PCA) and a dendrogram were conducted to explore the basic (dominant) components in species composition of all grazing intensities. PCA was based on the correlation matrix. For the analysis, all plant species participating in the composition of the grasslands with a percentage of 5% and above were used, covering the three years of measurements and all grazing intensities. The statistical package vegan, part of R [45], was employed for the analysis.

3. Results

3.1. Plant Cover and Species Composition

Plant cover was lower and bare soil was significantly higher in the highly grazed area for the three years of measurements, compared to the areas with moderate grazing intensity and the ungrazed ones (p-values = 0.017 and 0.010). However, it must be emphasized that even in the highly grazed area, plant cover was relatively high (90.9%, 87.6%, and 94.9%) for 2013, 2014, and 2015, respectively. Grazing intensity overall did not significantly affect litter percentage (p-values = 0.161). However, the percentage of litter was significantly higher in the highly grazed area compared to the other two in year 2015 (Table 1).

Table 1. Means of vegetation cover (%) for three grazing intensities for the years 2013, 2014, and 2015.

	Grazing Intensity								
	2013			2014			2015		
Category	Highly grazed	Moderate grazed	Ungrazed	Highly grazed	Moderate grazed	Ungrazed	Highly grazed	Moderate grazed	Ungrazed
Plant cover (%)	90.9 b	95.9 a [1]	95.3 ab	87.6 a	91.1 a	91.3 a [1]	94.9 b	98.4 a	99 a [1]
Litter (%)	5.8 a	3.6 a	4,2 a	7.2 a	6.4 a	8.5 a	4.1 a	1.10 b	1.0 b
Soil (%)	3.3 a	0.5 b	0.5 b	5.2 a	2.50 ab	0.20 b	1.0 a	0.5 ab	0.0 b

[1] Means on the same line followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level.

The percentage of woody species was affected by grazing intensity and was significantly higher in the ungrazed area (p-values = 0.000), while woody species either did not participate at all or participated in a small amount to the grazed areas for the three years of measurements. The percentage of grasses and legumes was generally lower in the ungrazed area compared to grazed ones for the three years of measurements (p-values = 0.000 and 0.000). The percentage of forbs in the ungrazed area was statistically significantly higher (p-values = 0.001) in relation to the moderate and highly grazed area for the three years of the measurements. According to the results, the lack of grazing affects species composition, mainly by favoring the forbs and woody species (Table 2).

Table 2. Means of participation of functional groups (grasses, legumes, forbs, and woody) in species composition (%) for three grazing intensities for the years 2013, 2014, and 2015.

	Grazing Intensity								
	2013			2014			2015		
Functional groups	Highly grazed	Moderate grazed	Ungrazed	Highly grazed	Moderate grazed	Ungrazed	Highly grazed	Moderate grazed	Ungrazed
Grasses (%)	63.53 a [1]	64.00 a	28.90 b	59.15 a [1]	54.76 a	39.86 a	69.88 a	74.64 a [1]	50.44 b
Legumes (%)	16.05 a	11.23 a	7.50 a	12.24 a	8.44 b	0.65 c	17.65 a	4.16 b	1.72 b
Forbs (%)	20.41 b	23.17 b	40.77 a	24.61 a	34.58 a	34.19 a	12.36 b	19.36 ab	28.46 a [1]
Woody (%)	0.0 b	1.60 b	22.83 a	0.0 b	2.21 b	25.29 a	0.11 b	1.84 b	19.38 a [1]

[1] Means on the same line followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level.

PC1 and PC2 axes together accounted for 67.78% of the total variance (Figure 4). Plant species formed four distinct cycle groups. The species formed in the red circle represents the ungrazed area, the green circle is the highly grazed area, the blue circle is the moderately grazed area, and, finally, the creation of a new group in the purple circle. The presence of woody vegetation and bracken in species composition was pronounced in the ungrazed area (red color), with the dominant species being *Rubus sanctus* Schreb., *Rumex acetosella* L., and *Pteridium aquilinum* (L.) Kuhn. subsp. *aquilinum*. Bracken (*Pteridium aquilinum* (L.) Kuhn. subsp. *aquilinum*) contributed to the composition of the ungrazed area (average) by 2.48%, 4.09%, and 5.21% for the years 2013, 2014, and 2015, respectively, while woody vegetation contributed to the composition of the ungrazed area by 22.83%, 25.29%, and 19.38% for the years 2013, 2014, and 2015, respectively. Additionally, other species with a strong presence in the ungrazed area included *Agrostis canina* L. subsp. *canina*, *Thymus sibthorpii* Benth., *Anthoxanthum odoratum* L., and *Bromus squarrosus* L. subsp. *squarrosus*. Dominant species in moderate grazing intensity were *Vulpia muyros* (L.) C.C. Gmel., *Chrysopogon gryllus* (L.) Trin., *Bromus hordeaceous* L., *Festuca ovina* aggr., and *Rumex acetosella* L., while those recorded in the high grazing intensity were *Cynodon dactylon* (L.) Pers., *Lotus corniculatus* L., *Plantago lanceolata* L., and *Lolium perenne* L.

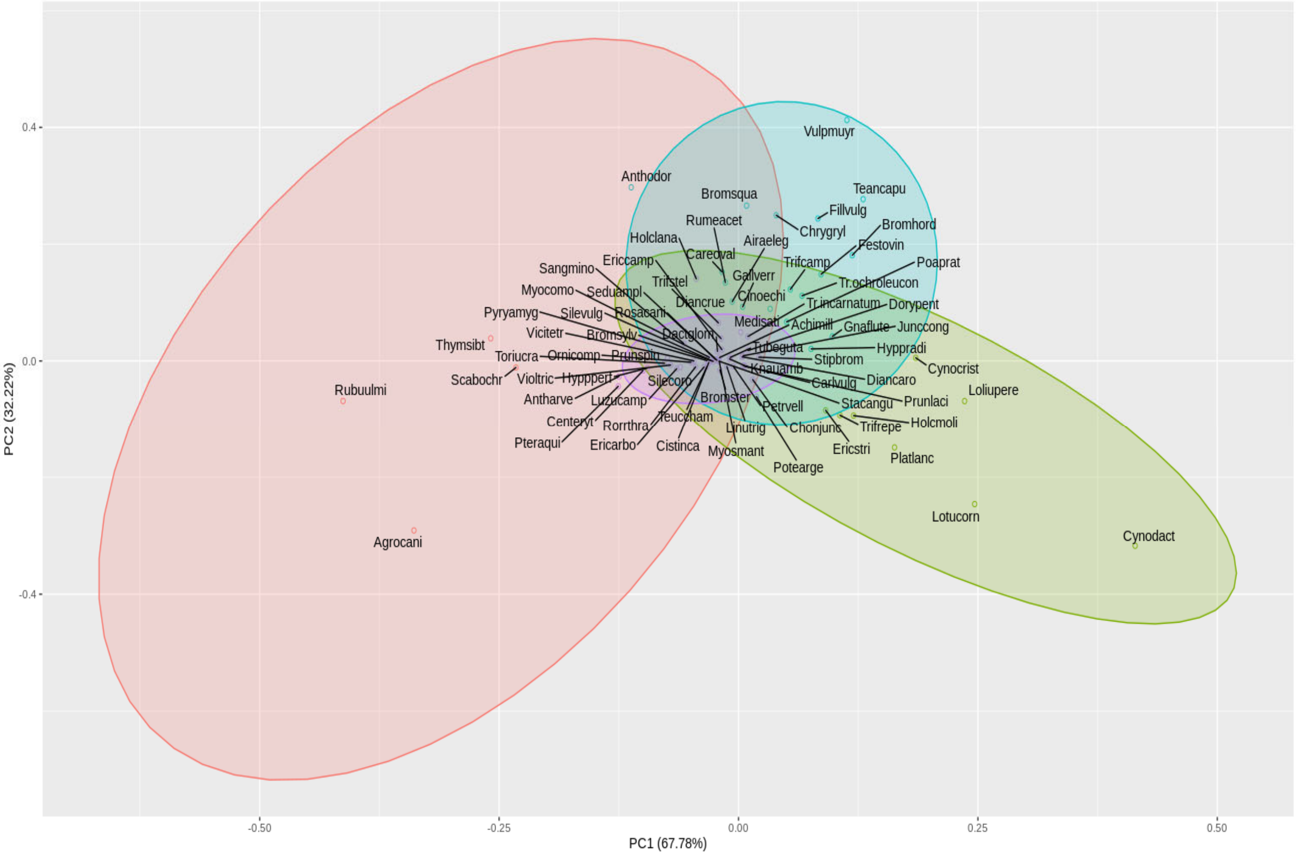

Figure 4. Principal component analysis (PCA) for the three grazing intensities based on species composition with a participation rate of at least 5%. PC1 and PC2 axes together accounted for 67.78% of the total variance. Plant species formed four distinct cycle groups. The species formed in the red circle represent the ungrazed area, the green circle is the highly grazed area, the blue circle is the moderately grazed area, and, finally, the creation of a new group in the purple circle. Antodor: *Antoxanthum odoratum* L., Vulpmuyr: *Vulpia muyros* (L.) C.C. Gmel., Bromsqua: *Bromus squarosus* L., Bromhord: *Bromus hordeaceous* L., Bromster: *Bromus sterilis* L., Rumeacet: *Rumex acetosella* (L.), Holclana: *Holcus lanatus* L. subsp. *lanatus*, Holcmoli: *Holcus mollis* subsp. *mollis*, Sangmino: *Sanguisorba minor* Scop, Ericamp: *Eringium campestre* L., Careoval: *Carex ovalis* Gooden, Airaeleg: *Aira elegans* Roem. and Schult, Chrygryl: *Chrysopogon gryllus* (L.) Trin., Fillvulg: *Fillipendula vulgaris* Moench., Teancapu: *Taeniatherum caput-medusae* (L.) Nevski, Trifcamp: *Trifolium campestre* Schreb.,

Trifochr: *Trifolium ochroleucon* Huds., Trifinca:*Trifolium incarnatum* L., Trifrepe: *Trifolium repens* L., Trifstel: *Trifolium stellatum* L., Festovin: *Festuca ovina* aggr., Poaprat: *Poa pratensis* L., Myossylv: *Myosotis sylvatica* Hoffm., Pyryamyg: *Pyrus spinosa* Forssk., Thymsibt: *Thymus sibthorpii* Benth., Rubuulmi: *Rubus sanctus* Schreb., Agrocani: *Agrostis canina* L. subsp. *canina*, Scabochr: *Scabiosa ochroleuca* L., Silevulg: *Lychnis vulgaris* (L.) Desr., Lotucorn: *Lotus corniculatus* L.,Cynodact: *Cynodon dactylon* (L.) Pers., Lolipere: *Lolium perenne* L., Cynocris: *Cynosurus cristatus* L., Platlanc: *Plantago lanceolata* L., Potearge: *Potentilla argentea* L., Myosmant: *Myosotis sylvatica* Hoffm., Cistinca: *Cistus creticus* L. subsp. *creticus*, Ericarbo: *Erica arborea* L., Pteraqui: *Pteridium aquilinum* (L.) Kuhn. subsp. *aquilinum*, Vicitetr: *Vicia tetrasperma* (L.) Schreb., Ericstri: *Euphrasia stricta* J.F. Lehm, Seduampl: *Sedum amplexicaule* subsp. *tenuifolium* (Sm.) Greuter, Rosacani: *Rosa canina* L., Bromsylv: *Brachypodium sylvaticum* (Huds) P. Beauv. subsp. *sylvaticum*, Toriucra: *Torilis ucranica* Spreng., Violtric: *Viola tricolor* L. auct. Fl. graec, Antharve: *Anthemis arvensis* L., Centeryt: *Centaurium erythraea* Rafm., Rorrthra: *Rorripa thracica* (Friseh) Fritch., Luzucamp: *Luzula campestre* (L.) D.C. subsp. *campestris*, Hyppperf: *Hypericum perforatum* L., Ornicomp: *Ornithopus compressus* L., Prunspin: *Prunus spinosa* L., Silecoro: *Lychnis coronaria* (L.) Desr., Teuccham: *Teucrium chamaedrys* L. subsp. *chamaedrys*, Dactglom: *Dactylis glomerata* L., Diancrue: *Dianthus cruentus* Griseb., Gallverr: *Gallium verum* L. subsp. *verum*, Cinoechi: *Cynosurus echinatus* L., Medisati: *Medicago sativa* (L.), Achimill: *Achillea millefolium* L., Gnaflute: *Helichrysum luteoalbum* (L.) Rchb., Stipbrom: *Achnatherum bromoides* (L.) P. Beauv., Tubeguta: *Tuberaria guttata* (L.) Fourr, Knauambi: *Knautia ambigua* Boss and Orth., Linutrig: *Linum trigynum* L., Petrvelu: *Petrorhagia velutina* (Guss) P.W., Carlvulg: *Carlina vulgaris* L. subsp. *vulgaris*, Chonjunc: *Chondrilla juncea* L., Stacangu: *Stachys angustifolea* M. Bieb., Prunlaci: *Prunella laciniata* (L.) L., Diancaro: *Daucus carota* L., Hyppradi: *Hypochaeris radicata* L., Junccong: *Juncus conglomeratus* L., Dorypent: *Dorycnium germanicum* (Gremli) Rikli.

The species that are associated with the fourth group (purple circle) mainly comprised forbs (*Moenchia mantica* L. Bartl., *Linum trigynum* L., *Potentilla argentea* L., *Chondrilla juncea* L., *Stachys angustifolia* M. Bieb., *Carlina vulgaris* L. subsp. *vulgaris*, *Knautia ambigua* Boss and Orth., *Daucus carota* L., *Juncus conglomeratus* L., *Achillea millefolium* L., *Dianthus cruentus* Griseb., *Sedum amplexicaule* subsp. *tenuifolium* (Sm.) Greuter, *Myosotis sylvatica* Hoffm., *Lychnis coronaria* (L.) Desr, *Torilis ucranica* Spreng., *Viola tricolor* L. auct. Fl. graec, *Hypericum perforatum* L., *Centaurium erythraea* Rafm, *Rorripa thracica* (Friseh) Fritch., *Teucrium chamaedrys* L. subsp. *chamaedrys*, and *Cistus creticus* L. subsp. *criticus*) in ungrazed and moderately grazed area. The listed species included in the above grazing intensities and grasses, such as *Bromus sterilis* L., *Achnatherum bromoides* (L.) P. Beauv., *Dactylis glomerata* L., and *Luzula campestris* (L.) D.C.; legumes, such as *Dorycnium germanicum* (Gremli) Rikli, *Trifolium incarnatum* L., *Medicago sativa* (L.), *Trifolium stellatum* L., *Ornithopus compressus* L., and *Vicia tetrasperma* (L.) Schreb.; and some woody species, like *Rosa canina* L., *Pyrus spinosa* Forssk, *Vicia tetrasperma* (L.) Schreb., *Prunus spinosa* subsp. *dasyphylla* (Schur) Domin, and *Erica arborea* L.

Out of these species, 12 are common in all three years of measurements, namely *D. cruentus* Griseb., *T. stellatum* L., *D. glomerata* L., *L. coronaria* (L.) Desr, *B. hordeaceus* L., *P. spinosa* subsp. *dasyphylla* (Schur) Domin, *O. compressus* L., *V. tricolor* L. auct. Fl. graec, *H. perforatum* L., *R. thracica* (Friseh) Fritch., *E. arborea* L., and *T. chamaedrys* L. (Supplementary Materials).

The results of the hierarchical clustering analysis are shown in Figure 5. The first group represents the ungrazed area for the years 2013, 2014, and 2015, suggesting that species composition for those years was similar and formed a distinct cluster. The second group primarily includes data from the years 2013 and 2014 based on moderate grazing intensity. This also indicates that species composition in these two years and under moderate grazing was similar and formed another distinct cluster. The third group mainly comprises data from the years 2013 and 2014 according to high grazing intensity. Similar to the previous group, this suggests that species composition in these two years and under high grazing intensity was similar and formed a separate cluster. The last group represented data from the year 2015, including both high and moderate grazing intensities.

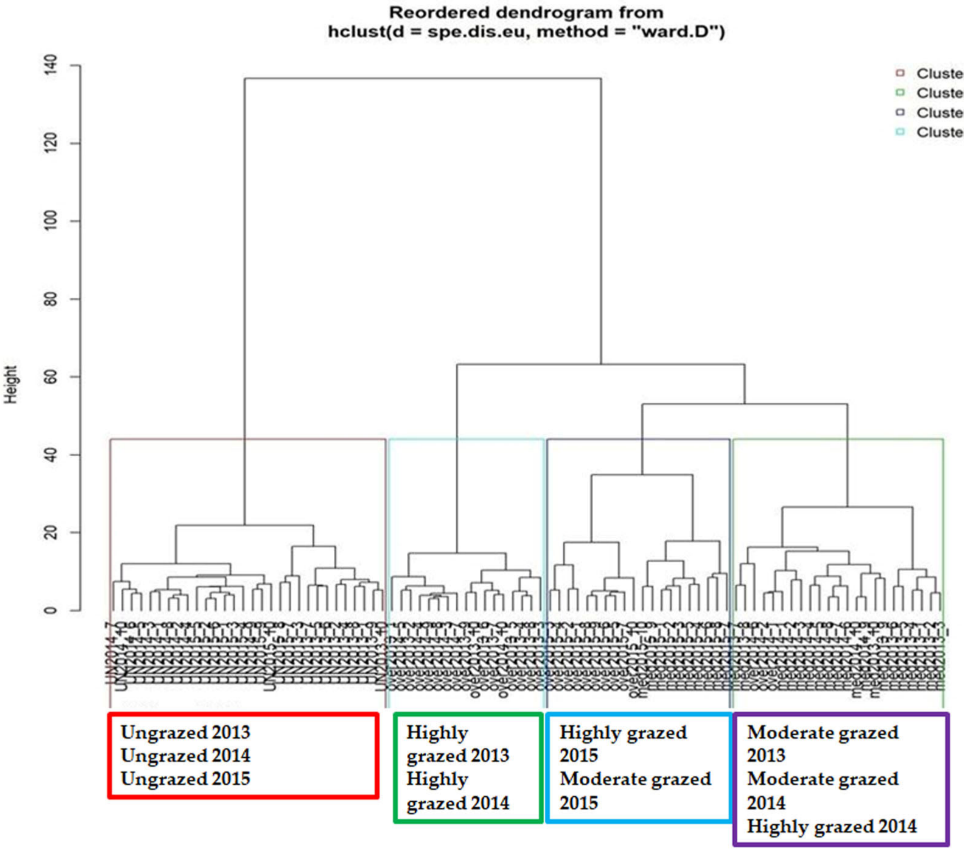

Figure 5. Dendrogram depicting the distance of plant species that participated in species composition at a rate of at least 5% for the three grazing intensities.

Table 3 shows the effect of grazing intensity on the plant cover of dominant species of the ungrazed area (ΔGE dc) for the three years of measurements (mean value). According to the results, *C. gryllus* (L.) Trin and *L. corniculatus* L. are affected differently in the two grazing intensities. *C. gryllus* (L.) Trin. increases its presence in moderate grazing intensity while it decreases its presence in high grazing. In contrast, *L. corniculatus* L. decreases under moderate grazing and increases under high grazing intensity. *A. canina* L. subsp. *canina* and *A. odoratum* L. decrease as grazing intensity increases, while all other species increase.

Table 3. Effect of grazing intensity on dominant species in the ungrazed area for the three years of measurements (2013, 2014, and 2015).

	ΔGEdc(M) [1]	**ΔGEdc(G)**
Chrysopogon gryllus (L.) Trin.	0.97	−0.54
Agrostis canina L. subsp. *canina*	−0.33	−0.82
Plantago lanceolate L.	0.72	0.82
Festuca ovina aggr.	0.85	0.74
Bromus hordeaceous L.	0.94	0.63
Trifolium repens L.	0.98	0.83
Cynodon dactylon (L.) Pers.	1.00	1.00
Lotus corniculatus L.	0.15	0.96
Lolium perenne L.	1.00	1.00
Anthoxanthum odoratum L.	−0.23	−0.61
Cynosurus cristatus L.	1.00	1.00

[1] G and UG are the coverage percentages of the species in the grazed and in the ungrazed area, respectively.

Table 4 presents the forage value index (FVI). The forage value index is used in order to assess the quality of forage for grazing animals. The highest FVI values are observed at high grazing intensity for the years 2013 and 2015. This suggests that, in these years, the vegetation provides the most favorable forage for animals. In contrast, in the year 2014, the highest FVI was associated with moderate grazing intensity, indicating that the vegetation in this area consisted of species with the highest quality for grazing animals. However, when you aggregate the data for the three years of measurements, FVI is the same (4.9) for both moderate and high grazing intensity implying that, when taking into account the overall forage quality across these three years, both moderate and high grazing intensity areas offer similar forage quality.

Table 4. Forage value index (FVI) for the three grazing intensities and the three years of measurements.

	Year of Measurements			
Grazing intensity	2013	2014	2015	Means
Ungrazed	3.1	2.8	3.6	3.2
Moderate grazed	4.7	5.0	4.9	4.9
Highly grazed	5.0	4.2	5.4	4.9

3.2. Floristic Diversity

Floristic diversity according to the Shannon–Wienner index was statistically significantly higher in moderate grazing intensity for the years 2013 and 2015 compared to ungrazed and highly grazed intensities (*p*-values = 0.000 and 0.002). In 2014, there were no statistically significant differences (*p*-value = 0.900) between the three grazing intensities (Table 5), although there was a tendency for higher floristic diversity in the moderate grazing intensity. The differences that exist between years can be attributed to different weather conditions (temperature and rainfall), which affect species composition and floristic diversity.

Table 5. Means of floristic diversity (Shannon–Wiener index) for the three grazing intensities for the years 2013, 2014, and 2015.

	Year of Measurements			
Grazing intensity	2013	2014	2015	Means
Ungrazed	1.40 a	1.46 a [1]	1.62 a [1]	1.49 a [1]
Moderate grazed	1.78 b	1.51 a	1.87 b	1.72 b
Highly grazed	1.45 a	1.50 a	1.85 b	1.60 b
Means	1.56 b	1.49 b	1.78 a [1]	

[1] Means on the same column followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level.

As it concerns the Simpson index, floristic diversity was statistically significantly higher in the moderate grazing intensity (*p*-values = 0.000, 0.015, and 0.005) for all years of the measurements compared to the other two grazing intensities, in which the differences were also statistically significant for the year 2013, while in the years 2014 and 2015, the two other grazing intensities did not differ statistically significantly from each other (Table 6). Simpson's index, regardless of grazing intensity, had the lowest value in 2015 compared to the other two years. Indicatively, the closer the Simpson index is to zero, the greater the diversity tends to be.

Table 6. Means of plant diversity (Simpson index) for the three grazing intensities for the years 2013, 2014, and 2015.

	Year of Measurements			
Grazing intensity	2013	2014	2015	Average
Ungrazed	0.37 a	0.33 a [1]	0.27 a	0.32 a [1]
Moderate grazed	0.23 c	0.26 b	0.22 b	0.24 b
Highly grazed	0.28 b	0.28 a	0.21 b	0.26 b

[1] Means on the same column followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level.

Eveness (J) showed the highest value at high grazing intensity for all three years and differed statistically significantly (*p*-values = 0.002, 0.004, and 0.004) (Table 7). The closer to one the value of evenness is, the more similar the ecosystem is considered to be, in terms of plant species.

Table 7. Means of evenness of plant species (J) in the three grazing intensities for the years 2013, 2014, and 2015.

	Year of Measurements			
Grazing intensity	2013	2014	2015	Average
Ungrazed	0.72 a	0.73 a [1]	0.79 a [1]	0.75 a [1]
Moderate grazed	0.75 a	0.72 a	0.79 a	0.75 a
Highly grazed	0.80 b	0.80 b	0.82 b	0.81 b
Average	0.76 ab	0.75 a [1]	0.80 b	

[1] Means on the same column followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level.

The number of species (S) and individuals per plot (N) were statically significantly higher in moderate grazed areas compared to highly grazed and ungrazed ones for the three years of measurements (Table 8) (*p*-values (S) = 0.000, 0.003 and 0.001, *p*-values (N) = 0.000, 0.000 and 0.022). Additionally, in 2015, the number of species and individuals of the species per plot showed higher values compared to years 2013 and 2014 for all grazing intensities.

Table 8. Means of the number of species (S) and number (N) of individuals per plot for the grazing intensities for the years 2013, 2014, and 2015.

	Year of Measurement			
Grazing intensity	2013	2014	2015	Mean
Ungrazed	7.48 (62) b	7.95 (46) ab	8.55 (44) b	7.99 b
	64.06 b	51.45 b	80.75 b	65.42 b
Moderate grazed	11.40 (56) a	8.83 (74) a	11.28 (48) a	10.50 a
	88.08 a	71.83 a	101.93 a	87.28 a
Highly grazed	6.45 (29) b	6.85 (41) b	9.70 (39) b	9.84 a
	51.13 c	59.70 b	93.12 ab	91.93 a
Mean	8.44 b	7.87 b	9.84 a	
	67.76 b	60.99 b	91.93 a	

Means on the same column followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level. Number of species per plot. Number of individuals of species per plot.

The Jaccard similarity index for the years 2013, 2014, and 2015 is presented in Table 9. Moderate grazing intensity had a higher similarity with high grazing intensity for the three years of measurements, while the ungrazed and the highly grazed had the lowest similarity for the years of measurements. During the years 2014 and 2015, the moderate grazed area showed high uniformity in species composition with the highly grazed area.

Table 9. The Jaccard similarity index between different grazing intensities (ungrazed, moderately grazed, and highly grazed) for years 2013, 2014, and 2015.

2013	**Ungrazed**	**Moderate Grazed**	**Highly Grazed**
Ungrazed	-	0.396	0.22
Moderate grazed	0.396	-	0.392
Highly grazed	0.22	0.392	-
2014	Ungrazed	Moderate Grazed	Highly Grazed
Ungrazed	-	0.367	0.278
Moderate grazed	0.367	-	0.588
Highly grazed	0.278	0.588	-
2015	Ungrazed	Moderate Grazed	Highly Grazed
Ungrazed	-	0.397	0.338
Moderate grazed	0.397	-	0.631
Highly grazed	0.338	0.631	-

As it concerns β-diversity for the three years of measurements, the ungrazed and moderate grazed area had a statistically significantly higher value compared to the highly grazed area (p-values = 0.001, 0.001, and 0.001) (Table 10).

Table 10. Means of β-diversity at the three grazing intensities for the three years of measurements (2013, 2014, and 2015).

	Year of Measurements		
Grazing intensity	2013	2014	2015
Ungrazed	5.36 a	5.69 a	5.99 a
Moderate grazed	4.94 a	5.74 a	5.18 a
Highly grazed	3.53 b	4.71 b	4.09 b

Means on the same column followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level.

3.3. Functional Traits of Plant Species

3.3.1. Specific Leaf Area, Leaf Dry Matter Content, and Shoot Dry Matter Content

Specific leaf area (SLA) was statistically higher in the ungrazed area compared to grazed ones (Table 11). On the other hand, leaf dry matter content (LDMC) was statistically lower in the ungrazed area compared to the areas that were grazed. Stem dry matter content (StDMC) presented its higher value in the moderately grazed area compared to the ungrazed and highly grazed areas but these differences were not statistically significant (Table 11).

Table 11. Means of specific leaf area (mm^2/mg) (SLA), leaf dry matter content (mg/g) (LDMC), and shoot dry matter content (mg/g) (StDMC) of the dominant species for the three grazing intensities.

Grazing Intensity	**SLA**	**LDMC**	**StDMC**
Ungrazed	345.40 a	65.66 b	327.30 a
Moderate grazed	220.74 b	339.42 a	352.24 a
Highly grazed	225.55 b	322.70 a	344.67 a

Means on the same column followed by the same letter are not statistically significantly different at the $\alpha = 0.05$ level.

3.3.2. Vegetative and Reproductive Height

Vegetative height (VH) and reproductive height (RH) were higher in the ungrazed area compared to the grazed ones. The lowest value was recorded in the highly grazed area (Table 12).

Table 12. Means of vegetative (VH) and reproductive (RH) plant height (cm) of dominant species for the three grazing intensities.

Grazing Intensity	VH	RH
Ungrazed	26.66 a	41.70 a
Moderate grazed	14.90 b	38.84 a
Highly grazed	2.19 b	8.71 b

Means on the same column followed by the same letter are not statistically significantly different at the α = 0.05 level.

3.3.3. Leaf Nitrogen and Phosphorus Concentration

In the ungrazed area, the leaf nitrogen concentration (LNC) was 1.24 mg/g, in the moderately grazed it was 1.29 mg/g, and in the highly grazed it was 1.7 mg/g but they did not differ statistically. Similarly, leaf phosphorus concentration (LPC) did not statistically differ among the grazing intensities (Table 13).

Table 13. Leaf nitrogen (LNC) and phosphorus (LPC) concentration (mg/g) at the three grazing intensities.

Grazing Intensity	N	LNC	LP
Ungrazed	10	1.24 a	0.58 a
Moderate grazed	10	1.30 a	0.51 a
Highly grazed	10	1.70 a	0.76 a
Mean	10	1.35	0.57

Means on the same column followed by the same letter are not statistically significantly different at the α = 0.05 level.

3.3.4. Qualitative (Categorical) Traits

The biotic form, life cycle, and photosynthetic pathway were the qualitative traits of the dominant species that were measured. The biotic forms of the dominant species included therophytes, hemicryptophytes, phanerophytes, and hamaephytes. Figure 6 depicts the number of groups for each of the three different grazing intensities. In the ungrazed area, all groups appeared but hemicryptophytes were the most abundant consisting of the species *L. campestris*, *A. canina* L. subsp. *canina*, *P. lanceolate* L., *S. minor* Scop., and *H. perforatum* L. Phanerophytes had the lowest participation with the species *R. sanctus* Schreb. Increased grazing intensity had as a result, especially in the moderate grazed area, namely the disappearance of phanerophytes, while the hemicryptophytes increased in number (10), and also the therophytes (9). In highly grazed areas, hemicryptophytes were the biotic form that was only found and was compromised of the species *P. lanceolate* L., *H.* L. subsp. *lanatus*, *A. canina* L. subsp. *canin*a, *L. perenne* L., *C. dactylon* (L.) Pers, *L. corniculatus* L., and *C. cristatus* L.

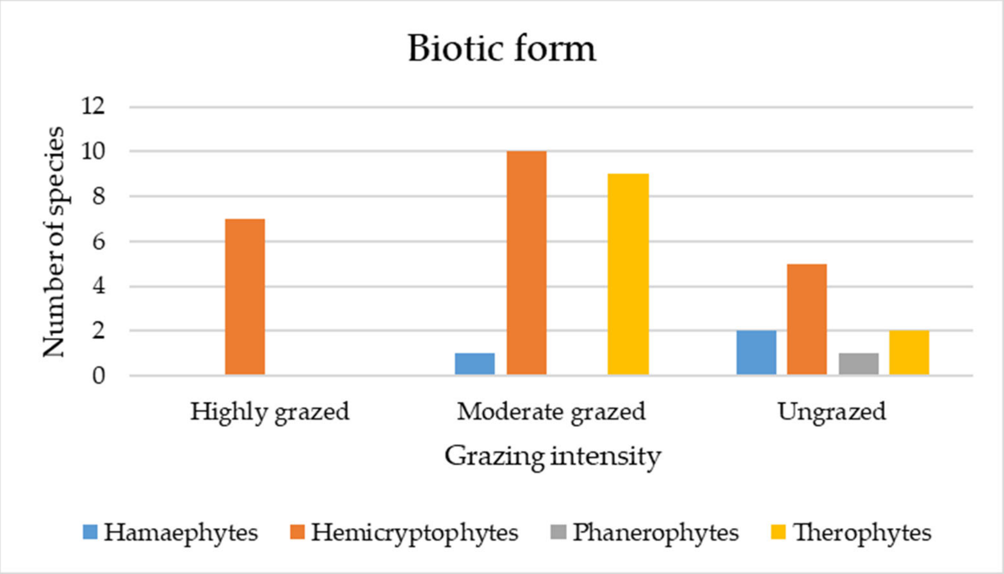

Figure 6. Distribution of species according to their biotic form.

In total, 1 out of the 10 species (*A. arvensis* L. and *T. gutata* (L.) Fourr) followed the annual life cycle in the ungrazed area, 9 out of 20 species in the moderate grazed area (*T. incarnatum* L., *T. ucranica* Spreng, *G. verum* L., *C. cristatus* L., *V. muyros* (L.), *B. hordeaceous* L., *L. trigynum* L., *C. echinatus* L., and *G. luteoalbum* (L.)), and 1 out of 7 species (*C. cristatus* L.) in the highly grazed area (Figure 7).

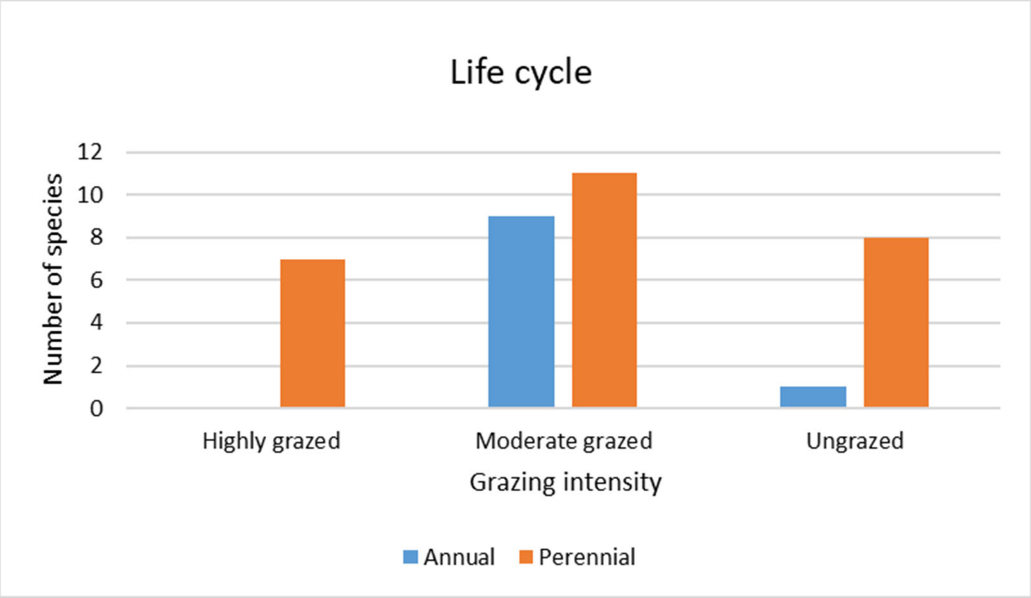

Figure 7. Distribution of species according to their life cycle.

The majority of species followed the C3 photosynthetic pathway, while only two grasses, *C. gryllus* (L.) Trin. And *C. dactylon* (L.) Pers., followed the C4 photosynthetic pathway (Figure 8).

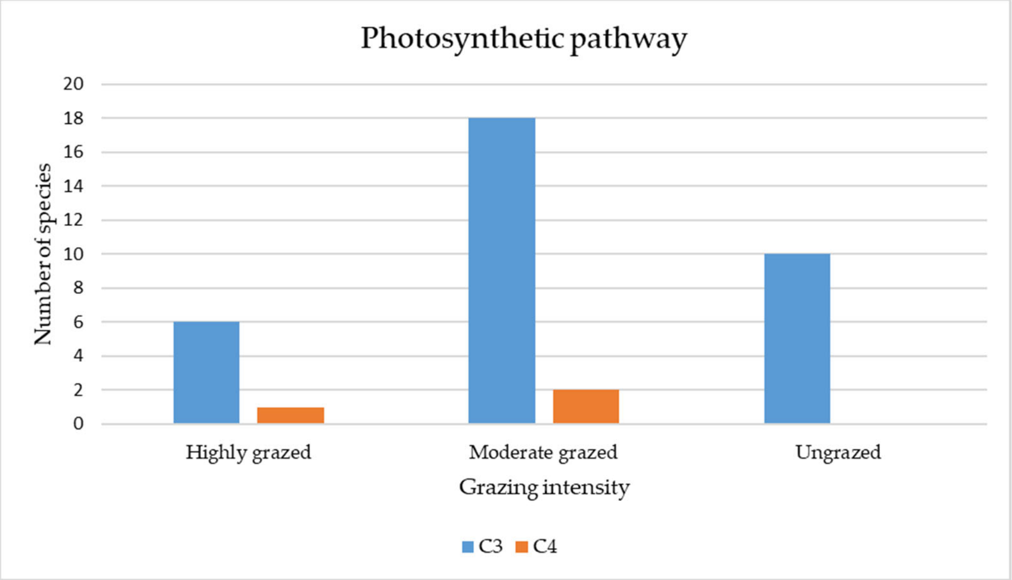

Figure 8. Distribution of species according to their photosynthetic pathway.

Also, the majority of species presented some defense mechanisms against grazing mainly by hairs, spines, and felt at all the grazing intensities (Figure 9).

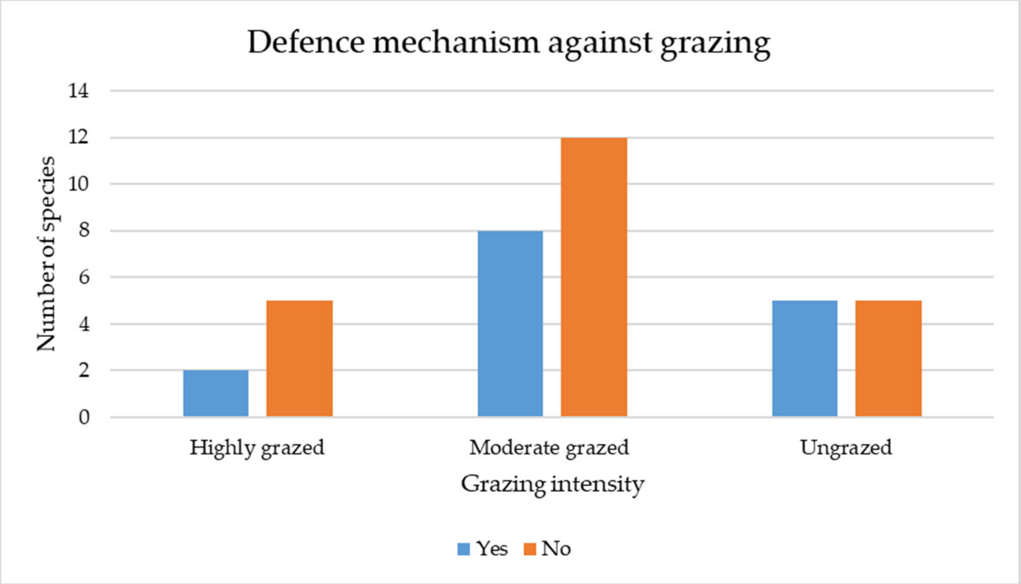

Figure 9. Distribution of species according to their defense mechanism against grazing.

4. Discussion

4.1. Plant Cover and Species Composition

The percentage of plant cover and bare soil was affected by grazing intensity and the year of measurements. The lowest percentage of plant cover and the highest of bare soil were recorded in the area that was highly grazed for all the years of measurements. Additionally, the lowest percentage of plant cover was recorded in 2014, the second year of measurements. The year effect is probably related to the variation in weather conditions from year to year, mainly in temperature and precipitation, during the growing season. From this point of view, 2014 was the year with the lowest precipitation in this particular period (May and June) (Supplementary Materials). Consequently, high intensity of grazing reduces plant cover and this negative effect is amplified in periods of drought.

Souther et al. [46], in a study that was conducted in semi-humid grasslands in Colorado considering how the long-term effect of grazing affects plant cover, concluded the same results as in the present study. In a meta-analysis by Eldridge et al. [47], conducted with data from grasslands of arid, semi-arid, humid, and semi-humid regions, concluded that the effect of grazing on plant cover was strong in arid and semi-arid areas, while it was relatively mild in humid and semi-humid areas. Determinant factors for the intensity of the grazing effect were the weather conditions and especially the variation in the percentage of precipitation from year to year. This is also confirmed in the present study. Even in highly grazed areas, plant cover was particularly high. Additionally, the smooth relief of the area may also have contributed to this.

The year of measurements and the intensity of grazing affected species composition in grasslands in the study area. Regarding the effect of years, the high amount of precipitation during the months of May and June 2015 combined with the high amount of precipitation during the autumn and winter of the previous year, seems to favor grasses, whose participation rate increased at all grazing intensities (Supplementary Materials). On the contrary, in the same year, the percentage of forbs in species composition decreased. It appears that there is a consistent pattern where species composition in the year 2015, regardless of grazing intensity (high or moderate), is similar. This might indicate that species composition or ecological factors in 2015 led to vegetation that was less affected by grazing intensity compared to the patterns observed in 2013 and 2014. Weather conditions are varying among the years and are considered a factor that strongly influences species abundance and composition [47,48]. Additionally, weather conditions have a more decisive role than grazing in the species composition of certain grassland ecosystems [48]. However, there are reports according to which weather conditions had no effect on species composition [49].

The decline in woody species in areas grazed by goats, such as in the study area, is expected. Goats generally prefer woody species [50–52], which participate in high percentages in their diet [53]. A similar decrease in woody species participation with the increase in grazing intensity is reported in both semi-arid [54–56] and semi-humid [57] grasslands. Grasses appear to be favored in the grazed areas, while forbs are favored in the long-term ungrazed areas. Legumes generally had low rates of participation in species composition, with a lower presence in the ungrazed area compared to the grazed one. Souther et al. [46] also reported similar results with the absence of grazing in semi-humid grasslands in Colorado about increasing forbs and also Scott-Shaw and Morris [58] in South Africa. On the contrary, in different geographical areas and climatic zones, it is reported that as grazing intensity increases, forbs increase and grasses decrease [59–62]. The dominance of grasses in the grazed areas in the present study (Table 2) could be attributed to a combination of factors related to the diet of the grazed animals (sheep and goats), the resistance of plant species to grazing, and their interactions with other species of the plant community and also with ecological factors such as soil properties. Additionally, in a study conducted by Abraham et al. [63] in the same area, it is reported that grass abundance is associated with high soil N content, which is also associated with high grazing intensity [64].

As they concern species response index to grazing (Table 3), the results showed that *A. canina* L. subsp. *canina* and *A. odoratum* L. decrease as grazing intensity increases, *C. dactylon* L. (Pers.), *L. perenne* L., *C. cristatus* L., *L. corniculatus* L., *P. lanceolata* L. and *T. repens* L. are favored by grazing, while *C. gryllus* (L.) Trin. is favored in moderate grazing intensity but decreased in high grazing intensity. *L. perenne* L. is concerned as a trampling and grazing tolerant species [65,66] as is *C. cristatus* L. [66]. Also, *C. dactylon* (L.) Pers. has creeping growth and is reproduced by stolons and rhizomes, characteristics that confer resistance to grazing [67]. Regarding *A. canina* L. subsp. *canina*, there is no literature related to its resistance to grazing. Among *Agrostis* species, *A. stolonifera* is reported as resistant to grazing [66] while *A. capillaris* is reported as susceptible [65] and/or resistant [68,69]. *L.*

corniculatus L., *P. lanceolata* L., and *T. repens* L. have morphological and reproductive characteristics that make them resistant to grazing.

Changes in species composition occur through grazing selectivity, which is generally reported to lead to a reduction in desirable grazing species and their replacement by other less desirable or undesirable species [59,60].

4.2. Floristic Diversity

Moderate grazing intensity appears to enhance floristic diversity in semi-humid grassland ecosystems according to the results of the present study. Similar results were reported by Torok et al. [70], in a study carried out in different types of grassland ecosystems in Hungary. The Shannon index and the number of species had their highest value at moderate grazing intensity.

In a study carried out in a steppe in China by Ren et al. [71], it was reported that floristic diversity based on the Shannon–Wienner index was not affected by grazing intensity. Skornik et al. [72], in a study conducted in grasslands in the Northern Adriatic at four grazing intensities, recorded the highest number of species at light grazing intensity and the lowest at high grazing intensity. On the other hand, according to Zhang et al. [73], floristic diversity based on the Simpson index increased with increasing grazing intensity in alpine grassland ecosystems in Qinghai Province, China. Similarly, Haarmeyer et al. [74] documented an increase in species abundance under high grazing intensity in grasslands in South Africa.

According to previous studies, the highest floristic diversity is recorded under light or moderate grazing intensity and any increase in grazing intensity has a negative effect on species richness. A meta-analysis of 116 published papers [75] regarding the effect of grazing intensity on species abundance and the Shannon–Wienner diversity index in temperate grasslands reported the same results. The authors mentioned that the results of the meta-analysis are consistent with the intermediate disturbance hypothesis.

A long-term absence of grazing (more than 40 years) in the present study appears to have reduced floristic diversity. Similar results were reported by Song et al. [76] in alpine grasslands in China where the short-term (2–4 years) and long-term (9–11 years) abandonment of grazing was also studied. Plant diversity based on the Simpson index in short-term grazing exclusion remained stable, while in the long-term, grazing exclusion decreased. Also, a decrease in the number of species was recorded in the mountainous grasslands of Slovakia after 46 years [77] and 10 years of abandonment of grazing [72]. Also, in a study conducted by Wang et al. [78], the abundance of species declined after five years of grazing exclusion in grasslands on the Tibetan Plateau. In contrast, in a meta-analysis performed by Xiong et al. [79], short-term exclusion of grazing (<5 years) increased the number of species in alpine and temperate steppes but nothing similar was observed in alpine grasslands. Grazing in natural ecosystems causes changes in the composition and structure of vegetation [15,36,80,81] and finally in floristic diversity.

The intermediate disturbance hypothesis (IDH) [82–84] has been widely used to describe the relationship of disturbance, such as grazing, with the floristic diversity of natural ecosystems. According to the hypothesis, moderate grazing intensity results in higher floristic diversity compared to the absence of grazing or high grazing intensity [13,84]. However, the conflicting results regarding the effect of grazing on the floristic diversity of grasslands indicate that the IDH does not hold true in all cases and the impact depends on various factors such as weather conditions, grazing history, type of grazing animal, and ecosystem productivity [13,15]. According to a meta-analysis by Gao and Carmel [85], IDH applies only to wet and semi-wet areas- like those of the study area, while it does not apply to dry areas. Furthermore, their analysis suggests that the absence of grazing reduces floristic diversity in wet and semi-wet areas while increasing it in dry areas. This could be attributed to differences in vegetation structure and species composition. Thus, in wet and semi-wet grasslands of the Mediterranean region, such as those in the study area, vegetation is relatively dense, with high plant growth rates, and mainly dominated by tall to medium-height grasses. Such species were also dominant in the study area's grasslands (see Supplementary Material). Competition for light is intense in these plant communities and grazing may contribute to reducing it and creating openings where less competitive species can establish, such as low grasses and forbs [22].

This effect is more intense under moderate grazing, resulting in an increase in floristic diversity [36]. On the contrary, the abandonment of grazing leads to the dominance of more competitive species results in the decrease in floristic diversity. Also, floristic diversity was affected by the year of measurements. The effect of the year refers to the differentiation in weather conditions from year to year, mainly temperature and precipitation. Ren et al. [71] in Mongolian steppes, Moradi et al. [86] in Iran, Xiong et al. [79], and Wu et al. (2012) in alpine meadows in China reported that floristic diversity was more affected by weather conditions than by grazing intensity. Conversely, Yan et al. [87] reported that the floristic diversity of grasslands in Mongolia was not affected by weather conditions (rainfall and temperature) but by grazing intensity. According to recent reports, the floristic diversity of grasslands is mainly affected by the amount of rainfall [88] and lesser by temperature. A study that took place in Tibet in 116 grassland sites indicates that species richness and Shannon diversity index are positively related to precipitation [88]. Furthermore, precipitation during the growing season appears to have a greater impact compared to the total annual precipitation [71,86]. Accordingly, the results of the present study indicate that higher diversity based on Shannon–Wiener, Simpson indices, and species richness (regardless of grazing intensity) was recorded in 2015, which was the year with the highest precipitation during the growing season (Supplementary Materials).

The Jaccard similarity index showed the highest value in highly grazed grassland compared to the ungrazed area and the one with moderate grazing intensity. A meta-analysis of Liu et al. [89] and Li et al. [90] for different grassland ecosystems, including semi-humid ones, concluded that the Jaccard similarity index is higher in grazed sites compared to the ungrazed ones. This is mainly attributed to the dominance of the most competitive species in the ungrazed grasslands. On the other hand, Ren et al. [71] in grasslands in Mongolia and Yan and Lu [91] in alpine grasslands in the Tibetan Plateau did not observe any variation in the Jaccard similarity index between grazed and ungrazed areas. Similarly, Pueyo et al. [92] in grasslands in northern Spain and at six grazing intensities observed no change in the Jaccard similarity index but a decreasing tendency when increasing grazing intensity.

In the semi-humid grasslands such as in the study area, the lowest value for β-diversity was recorded in highly grazed areas, indicating a greater homogeneity of plant communities compared to the ungrazed ones. This tendency was not observed in moderate grazed areas. This may be attributed to different degrees of environmental differentiation (soil, micro-topography, etc.) of the areas with moderate and high grazing intensity. How grazing affects plant community composition and floristic diversity depends on many environmental and management factors. In addition to these factors, the small-scale differences of each area play a decisive role in which way grazing will affect the plant community, i.e., the homogeneity or heterogeneity in terms of soil, microtopography, etc. If those differences are small, the role of grazing is decisive and there is a tendency to homogenize composition [91,92]. On the contrary, when those differences are great, the role of grazing is then limited.

In conclusion, the results of the present study are consistent with the intermediate disturbance hypothesis [84], since all diversity indices were superior at moderate grazed areas. However, these changes in floristic diversity seem to be influenced and varied by the weather conditions of each year as well.

4.3. Functional Traits of Plant Species

Specific leaf area (SLA) in the plant community presented its highest values in the ungrazed area, compared to the grazed ones. This practically means that the plants in the ungrazed area tended to have larger leaves with lower weight. In contrast, leaf dry matter content (LDMC) and stem dry matter content (StDMC) presented their lowest value in the ungrazed area compared to grazed areas. However, it must be emphasized that stem dry matter content (StDMC) is a functional trait that has not been particularly studied and the literature references were limited [93]. Similarly, a trend of increasing specific leaf area in wet ungrazed grasslands in Southwest Germany was also observed by Kahmen and Poschold [94] and Wang et al. [95], in semi-arid grassland ecosystems in China. As it concerns LDMC, the results of this research are in agreement with those of Louault et al. [96] and Cruz et al. [97] in wet grassland ecosystems in France and Brazil, respectively. Additionally, Jascke et al. [98], in a study carried out in semi-arid grassland ecosystems in the Tibetan Plateau and in two grazing intensities (high and light), did not observe any difference in SLA due to grazing intensity as Peco et al. [99] in semi-arid grassland ecosystems in central Spain.

The higher value of SLA in the ungrazed area can be attributed to plant species' strategy in order to absorb more solar radiation, under shade conditions, created due to the density of the plant community [99–101]. Lower values of SLA at highly grazed areas are related to greater leaf resistance [15] and reduced palatability [100], which is also a strategy in order to avoid grazing.

In contrast, Streit et al. [102], in dry grasslands in southern Brazil, reported a decrease in SLA in the ungrazed area, while LDMC did not show any clear tendency related to grazing intensity. Golodets et al. [103], in semi-arid grasslands in northern Israel, reported that SLA and LDMC had their lowest values in the ungrazed area (area protected from grazing). However, a decrease in SLA in the ungrazed area and an increase in LDMC are reported in a number of studies [15,99,104,105]. The changes occurring in the above traits after the abandonment of grazing indicate that the photosynthetic rate of plants decreased and that their growth rate retarded [105].

SLA and LDMC depict plant strategies for absorbing nutrients from the soil and then utilizing them appropriately. Contrasting results presented in the aforementioned studies could be explained by the different grassland habitats that were examined (arid and semi-arid), the various species within the plant communities, and the distinction in the survival strategies employed by the various species within the plant community [102]. After long-term exclusion of grazing, there is a spread of woody vegetation, increasing the density of plant cover and reducing available solar radiation to some small plant species, which attempt to develop their leaves [102]. Conversely, plant species under high grazing intensity produce a greater number of leaves in order to compensate for the loss of leaf mass. Thus, plant communities subject to long-term and high-intensity grazing are characterized by plant species with small surface areas and large leaf masses in semi-arid grassland ecosystems [95]. These grazing intensity responses facilitate the ability of each plant to acquire resources to recover its losses and ensure its growth [41] and thus directly reflect the ability of plants to adapt to the environment [105].

The vegetative (VH) and reproductive height (RH) of the species presented their highest value in the ungrazed area compared to grazed ones. Plant height is considered as the functional trait that can best predict the response of plants to grazing [22,106]. Generally, the studies show that plant height increases in the ungrazed area [15,21,94,95,98,99,104,105,107]. Grime [108] reported that the increase in plant height in the ungrazed area is related to the competition of plants for light in order to survive.

The leaf nitrogen (LNC) and leaf phosphorus concentration (LPC) of the plants in the present study had the tendency for higher values in the highly grazed area compared to the other two areas. Janscke et al. [98] and Wang et al.[105] reported the same results regarding leaf nitrogen concentration studying semi-arid grassland ecosystems in China. However, Golodets et al. [103], in semi-arid grassland ecosystems in northern Israel, observed that leaf phosphorus concentration increased in the ungrazed area while leaf nitrogen concentration was not affected by grazing intensity. The tendency for higher values in leaf nitrogen concentration at high grazing intensity may be due to the presence of animals that, with their urine and feces, increase the soil nitrogen content [89,109]. Also, grazing animals, as they move into the grasslands, accelerate the rate of decomposing dry foliage causing an increase in soil nitrogen [78]. Therefore, the increase in soil nitrogen may be the main reason for the increase in leaf nitrogen concentration. This increase in soil fertility due to the presence of nitrogen also causes an increase in leaf phosphorus concentration [105,110]. Due to the increase in soil fertility, plant species develop their shoot at a faster rate and the concentration of phosphorus in the leaves also increases.

Hemicryptophytes predominated in the three grazing intensities. Castro et al. [104], in the grasslands of Portugal, reported that hemicryptophytes predominated in the ungrazed area. In fact, they emphasize that after a 15-year abandonment of grazing, the hamaephytes decreased, while after 30 years of abandonment of grazing, hemicryptophytes prevailed. Janscke et al. [98], in a study carried out in China, reported that no change in the biotic form of plants was observed in relation to grazing intensity. De Bello et al. [21], in another study, reported that phanerophytes predominated in ungrazed areas, mainly due to the increase in shrubs. However, this was not observed in the present study since phanerophytes appeared only in the ungrazed area. The presence of hemicryptophytes in the highly grazed areas is attributed mainly to special conditions of the mountainous grasslands since the combination of the high altitude with the climatic conditions (strong winds and cold winters) had, as a result, the seeds of the plant species found in dormancy, to be closer to or in the soil [111,112].

Perennial species dominated over annual species at all three grazing intensities. De Bello et al. [21], in grasslands in Spain and at three grazing intensities, reported that perennial species prevailed over annuals, after long-term exclusion of grazing. Bonet and Pasas [113] referred to the fact that moderate grazing intensity may favor annual species, which partly happened in the present research, although the percentage of participation of annual and perennial species was approximately the same. In contrast, Jascke et al. [98] reported that perennial species were not affected by grazing intensity, indicating that perennial species may not be so sensitive to grazing in dry grasslands such as those studied in their study (China), a finding that is also in agreement with the results of Díaz et al. [114]. In the present study, grasslands are considered as humid and according to Díaz et al. [114], in dry grasslands with a long history of grazing, the effect of grazing intensity does not significantly affect the proportion of annual and perennial species.

From the results of the present study, it appears that for all grazing intensities, the percentage of species that followed the C3 photosynthetic pathway was higher than those that followed the C4 photosynthetic pathway. Particularly, in the ungrazed area, all the species followed the C3 photosynthetic pathway. The results from the present study are in agreement with the results of Karakosta [115] in the same study area since all the species in the ungrazed area followed the C3 photosynthetic pathway. More generally, plants that follow the C3 photosynthetic pathway predominate in mountainous grasslands (higher altitudes) where temperatures are lower [3]. Cornelissen et al. [40] reported that compared to C3 plants, C4 plants tend to predominate in warm, sunny, and relatively dry environments.

The majority of species exhibited a defense mechanism against grazing across all three grazing intensities. However, in the ungrazed area, the presence or absence of a defense mechanism is almost the same. The density of the herbaceous layer, due to the intense presence of woody species, provides protection to herbaceous plants against grazing [116], allowing less adapted species to prevail. According to Papadimitriou [93], in the ungrazed area, taller species overshadow shorter ones, offering them protection from grazing. In areas with high grazing intensity, dominant species try to develop avoidance mechanisms, such as low height [22]. Additionally, the lower value of specific leaf area is likely associated with high leaf resistance and low palatability, serving as another defense mechanism against grazing in areas with high grazing intensity [93].

The study of plant functional traits facilitates the understanding of the survival and performance mechanisms of plants in different grassland ecosystems [117]. Additionally, useful conclusions about the response of grassland ecosystems to abiotic and biotic disturbances can be drawn through functional traits. Numerous studies indicate that plant biotic form and height are directly influenced by grazing intensity [21,98,106,118]. However, they emphasize that plant life form, height, and reproductive strategies can be considered examples of adaptation not only to grazing but also to climatic conditions. The combination of plant height, plant life form, and leaf mass is suitable for studying the response of species to grazing, while specific leaf area cannot be used as a trait response to grazing [119]. According to the present study, it appears that plant life form is not affected by grazing intensity, while plant height, specific leaf area, and leaf dry matter content can be used to study the response of plant communities to grazing in the studied grasslands.

5. Conclusions

The main disturbance in grasslands is grazing. For the mountainous grasslands such as those in the present study, the management goal must be the application of moderate grazing intensity. The absence of grazing for more than 40 years resulted in the invasion of shrubs, the increase in density of plant community, and decrease in floristic diversity. Tall competitive grasses dominated and legumes declined, which may have contributed to the reduction in nutritional value. Management measures must aim at enhancing species composition and then applying moderate grazing intensity. On the contrary, in highly grazed areas, sites with bare soil are observed, which also can lead to soil erosion. In this case, grazing animals should ideally be removed from those grasslands for a reasonable period of time in order to allow plant species to recover and then moderate grazing intensity must be applied.

In conclusion, grazing appears to be a crucial factor in shaping plant community and floristic diversity in grasslands. However, the way it influences their formation and the correlation between them is also influenced by many other factors such as climate, soil, and grazing history, which vary in each specific grassland. What should be further studied in these ecosystems is the role of climatic conditions, the specific topography, and the intensity of grazing in the study of plant functional traits. The combined study of the above levels of diversity can be a useful tool for exploring the intensity of grazing in grasslands and contributing to their sustainable management.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/land13040488/s1. Figure S1: Hyothermic diagram 1973–2015; Figure S2: Hyothermic diagram 2013; Figure S3: Hyothermic diagram 2014; Figure S4: Hyothermic diagram 2015; Table S1: Species composition list 2013-2014-2015; Table S2. Defense mechanism against grazing.

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