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Research Article

Assessment of quality and chemical composition of continental halophytic grasslands in south-east Europe

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Abstract

Continental halophytic grasslands are known for performing of range of ecosystem services especially remarkable in the regions where they are much distributed – in arid and semi-arid areas. Continental halophytic grasslands of the Central and South-East Europe are not considered as favourable for arable farming, however, traditional animal husbandry plays a crucial role in maintaining biodiversity and preserving these natural habitats. The particular interest of this study is the assessment of the chemical composition of biomass and the quality of insufficiently studied halophytic grassland communities of the central Balkans. In addition, the differences in pastoral value were monitored along the geographical gradient, i.e., between grasslands situated in the Pannonian plain (grasslands of Alliance *Puccinellion limosae*) and those distributed on the south Serbia (Alliance *Festucion pseudovinae*). The study was carried out at 18 representative sites, focusing on the saline grasslands of the two distinct regions – on the north and on the south of Serbia. The obtained results show that the halophytic grasslands have satisfying quality characteristics (average values: dry matter - 93.67%, crude protein - 8.66%, cellulose - 30.36%, crude fat - 2.27%) compared with other studied grasslands of saline habitats. There were no significant differences in quality and chemical composition of grasslands of the two regions, despite differences in floristic composition, indicating that salinity is the key determinant for pastoral value of the halophytic vegetation. The certain variations were attributed to the specific floristic composition related to ecological conditions and halophytic community characteristics.

Keywords: chemical composition; continental halophytic grasslands; suitability for utilization

Introduction

Halophytes represent a small group of plants which is widely dispersed within different plant families (Attia-Ismail, 2018) contributing for about 2% of the total terrestrial flora (Ben Hamed and Custodio, 2019). It has been estimated that up to one-third of halophytes, could be used as a forage (Aronson, 1985). Halophytic flora and vegetation are primarily associated to habitats containing concentration of the sodium salts in soil

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solution higher than 200 mM (Flowers and Colmer, 2008). Such habitats have widespread geographic distribution, covering about 7-10% of the Earth's land surface (Dudal and Purnell, 1986) and are especially present but not limited to arid and semi-arid regions. Halophytes may occur in nature in the form of trees or shrubs, but commonly they are grasses and forbs of many could serve as livestock forage and are a major food sources in animal feeding in case they are dominant flora of a certain region (e.g. Middle East, countries of North Africa, India, parts of Australia, etc.). The Pannonian halophytic grasslands are among the best-preserved grassland habitats in Europe, traditionally managed by moderate cattle and sheep grazing, as their poor soil quality and fluctuating water balance are unsuitable for intensive agriculture and forestry (Török *et al.*, 2011; Valkó *et al.*, 2014). Several studies also confirm active grazing and other types of use of European continental halophytic grasslands or in Mediterranean basin (Dajic-Stevanovic *et al.*, 2008; Dítětová *et al.*, 2016; Abd El-Hack *et al.*, 2018).

Recent studies on halophytic grasslands management, chemical composition and quality estimation of their potential as source for animal food have been conducted in the Middle East and North Africa (Egypt, Nigeria, Tunisia, Algeria), as well as in South Africa (Du Toit *et al.*, 2004; Attia-Ismail *et al.*, 2009; El Shaer and Attia-Ismail, 2015; Medila *et al.*, 2015; Moujahed *et al.*, 2015; Attia-Ismail, 2018; Hessini *et al.*, 2020; etc.). The management, conservation and traditional grazing of Central European alkali grasslands by cattle has been the subject of several studies (Molnár and Borhidi, 2003; Török *et al.*, 2014; Valkó *et al.*, 2014; Tóth *et al.*, 2018). However, there is insufficient information on the chemical composition and quality of inland halophytic vegetation, referring mainly to European region and its particular parts. The available studies on the quality of animal feed mostly targeted the chemical composition of selected plant species which are used as animal feed, such as those of genera *Atriplex, Kochia, Juncus, Suaeda, Salsola*, etc. In addition, individual halophytic species from coastal saline areas were screened for their nutritional quality (El-Shaer and Attia-Ismail, 2002), as well as some of terrestrial halophytes as a viable supplementary food source (Centofanti and Banuelos, 2019). It is known that species of the genus *Atriplex* are often used as an alternative animal feed, especially in conditions of deficiency of adder fodders, such as in early spring, during hot and dry summer months and late autumn (Osman *et al.*, 2006). The average value of the several studied halophytes from the arid regions refers to about 10% of total proteins, 21.7% of the crude fiber and 27% for ash (Abd El-Hack *et al.*, 2018). According to Temel *et al.* (2015), the majority of halophytes are underused due to insufficient knowledge about their chemical composition, as well as their potential nutritional value and yield. Moreover, it is known that salt affected soils are characterized by much lower fertility and other traits needed for production of traditional crops and therefore are mostly used for grazing (Kazemi and Eskandari, 2011). However, it is known that management of salt meadows involves grazing and mowing, where most of halophytic species have a satisfactory fodder value and are the basic resource for feeding domestic animals in drylands (Arzani *et al.*, 2010; Rad *et al.*, 2013; Oktay and Temel 2015).

In Europe, continental salt grasslands have a limited geographical distribution. Continental halophytic grasslands occur in the central Europe (Pannonian Plain) under strong influence of continental climate and in the southeast Europe (Balkan region, mainly southern parts of Serbia, Northern Macedonia and Bulgaria) under the influence of the sub-Mediterranean climate and are restricted to small patches. Besides climatic impacts, there are a number of ecological and geographical factors differentiating Pannonian and central Balkan halophytic grasslands, which primarily reflects on the floristic composition and vegetation types (Lukovic, 2019).

The focus of this study was the vegetation of the class *Festuco-Puccinellietea* and its two typical salt grassland alliances: *Puccinellion limosae*- Pannonian hypersaline open grasslands on solonetz and solonchack soils, composed of typical alkali grasses and *Festucion pseudovinae*- Pontic Pannonian salt-steppe pastures and saline steppic pastures on solonetz soils, composed of short grass communities dominated by short bunch and species tolerant to grazing and trampling (Borhidi *et al.*, 2012; Mucina *et al.*, 2016). These two alliances are common for Pannonian and central Balkan saline grasslands, while the occurrence of communities composed of succulent euhalophytes such as *Salicornia eurpaea*, *Suaeda maritima*, *Salsola soda* and some other highly tolerant species.

The aim of this paper is to present the chemical composition and pastoral value of the most distributed continental saline grasslands along the geographical gradient from the north (the Pannonian Plain) to the south of Central Balkans, and to investigate possible differences in the quality of grasslands of the two main halophytic alliances *Puccinellion limosae* and *Festucion pseudovinae.*

Materials and Methods

Study area

The studied area includes the continental Pannonian and the Central Balkan salt grasslands with focus on the territory of the Republic of Serbia. The vegetation of continental saline habitats is very well developed in the Pannonian Plain (Eliaš *et al.*, 2013; Lukovic, 2019), while towards the south, the occurrence of saline soils and euhalophytic vegetation decreases (Zlatković *et al.*, 2014). The sampling sites include 18 halophytic grasslands (13 on the territory of the Pannonian Plain and 5 in central Balkan) (Figure 1, Table 1). The plant material was collected for the chemical quality analysis. The sites were selected according to: 1. floristic and plant communities' diversity, 2. variety of salt soils (from wet to moderately wet salt marches and dry salt meadows), 3. subtle differences in soil chemical composition and 4. traditional management. Phytocoenological relevés were carried out at these selected sites in 2012, 2013 and 2014 according to the method of Braun-Blanquet (1964). The average size of the plots was $16m^2$. Plant material was collected by cutting the square plots of $1m^2$. The fresh plant material was measured *in situ*, while the dry biomass was measured after 7 days of drying at room temperature in the dark. The prepared material was grounded and stored in the dark, in well-sealed pots. Voucher specimens of plant species were deposited in the herbarium of the Department of Agrobotany, Faculty of Agriculture, University of Belgrade.

All phytocoenological relevés were stored in a vegetation database (Dajić Stevanović *et al.*, 2012, update 2015) using the TURBOVEG software (Hennekens and Schamineé, 2001). The affiliation of *relevés* to a particular vegetation type (Table 1, column 7) is determined based on the floristic composition and classification of halophytic vegetation in a larger dataset (Lukovic, 2019).

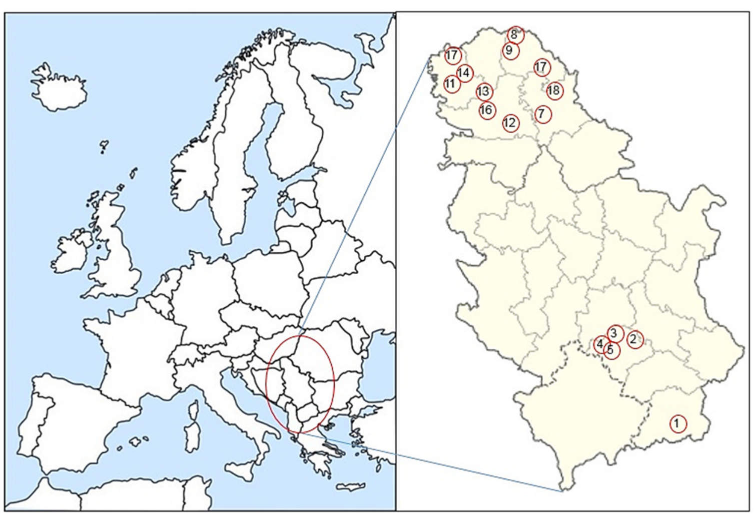

Figure 1. Geographical location of the studied area with the 18 selected sites

1. Aleksandrovačko jezero, 2. Lalinačka slatina, 3. Oblačinsko jezero, 4. Bresničić, 5. Suva česma, 6. Žabalj 1, 7. Žabalj 2, 8. Trešnjevac, 9. Gornji Breg, 10. Slano Kopovo, 11. Rančevo, 12. Bački Brestovac, 13. Ruski krstur, 14. Mali Stapar, 15. Novi bečej-Matej, 16. Despotovo, 17. Stanišić, 18. Melenci

Table 1. General information about the study sites

Site code	Releve No.		Sites	WGSe	Soil type	Type of vegetation (alliance)	Recorded management
L1	1624	Central Balkan halophytic grasslands	Aleksandrovačko jezero	N42.48843 E21.90290	Solonchak	*Puccinellion limosae*	Grazing
L2	1610		Lalinačka slatina	N43.34423 E21.76332	Solonchak	*Puccinellion limosae*	Abandoned, protected area management
L3	1620		Oblačinsko jezero	N43.30753 E21.68011	Solonchak	*Puccinellion limosae*	Grazing
L4	1631		Bresničić	N43.24740 E21.45213	Solonchak	*Puccinellion limosae*	Abandoned partly, grazing
L5	1628		Suva česma	N43.23305 E21.51188	Solonchak	*Puccinellion limosae*	Abandoned
L6	1516	Pannonian halophytic grasslands	Žabalj 1	N45.37965 E20.11410	Solonetz	*Festucion pseudovinae*	Grazing
L7	1518		Žabalj 2	N45.38068 E 20.16483	Solonetz	*Puccinellion limosae*	Grazeing
L8	1520		Trešnjevac	N 45.98589 E19.99917	Solonchak	*Puccinellion limosae*	Abandoned, grazing
L9	1524		Gornji Breg	N45.90302 E20.02411	Solonchak	*Festucion pseudovinae*	Abandoned
L10	1549		Slano Kopovo	N45.61734 E20.21955	Solonchak	*Festucion pseudovinae*	Grazing, protected area management
L11	1528		Rančevo	N45.86204 E19.12235	Solonchak	*Festucion pseudovinae*	Abandoned
L12	1530		Bački Brestovac	N45.63419 E19.31863	Solonchak	*Puccinellion limosae*	Abandoned, grazing
L13	1533		Ruski Krstur	N45.55832 E19.45683	Solonchak	*Puccinellion limosae*	Abandoned, grazing
L14	1539		Mali Stapar	N45.66444 E19.21706	Solonchak	*Puccinellion limosae*	Abandoned, grazing
L15	1536		Novi Bečej – Matej	N45.64985 E20.16335	Solonetz	*Festucion pseudovinae*	Grazing
L16	1543		Despotovo	N45.44177 E19.51827	Solonchak	*Puccinellion limosae*	Grazing
L17	1597		Stanišić	N45.92102 E19.15299	Solonchak	*Puccinellion limosae*	protected Grazing
L18	1535		Melenci	N45.52851 E20.29839	Solonetz-Solonchak	*Puccinellion limosae*	Grazing, area management

* The numbers in the first column refer to the numbers of phytocoenological relevés stored in the Turboveg database and correspond to Figure 2.

Chemical analysis

Chemical analysis of the main quality parameters of the plant material was carried out at the Institute for Fodder Plants in Kruševac (Serbia). Dried sampled plant material was analysed using the Weende analytical system: Hygroscopic moisture; Dry matter (DM); The content of raw ash was determined by dry incineration at 550 degrees; The content of crude protein (CP) was determined indirectly by the amount of total nitrogen by multiplying it by the factor 6.25, the Bremner modification (Bremner *et al.*, 1965); crude cellulose (C) content was determined by successive analysis of the sample with a diluted solution of H_2SO_4 in NaOH

(Henneberg and Stohman, 1859); crude fat content (CF) was determined by Soxhlet extraction; BEM- free nitrogen extracts were obtained by subtracting 1000 g dry matter from the sum of: Crude ash, Crude fat, Crude protein and Crude cellulose; Determination of cellulose fractions of ash free neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined by the method of Van Soest (1984).

Statistical data analysis

Since the sample size was too small to calculate statistical differences between the analysed groups, a non-parametric Mann-Whitney U test was performed. Data were analysed using the SPSS 26.0 software (SPSS, IBM Corp., 2019). Floristic richness (number of species per *relevé*) calculated using the Shannon-Wiener (H) diversity index in Juice software version 7.1 (Tichý, 2002). For species selection within biological groups, as well as within alliances, coverage coefficient expressed on a scale (r, +, 1-5) was used. Only the species with estimated coverage higher than 1 were considered (Figures 3, 4). The results of comparison between groups were visually displayed using the Population Pyramid histogram and level of similarity between groups calculated using the Jaccard index. The results of chemical analysis of halophytic grassland samples were graphically presented using the ordinal analysis method (DCA) in CANOCO (Braak and Smilauer, 2002).

Results

Phytocoenological and floristic characteristics of the studied areas

The halophytic vegetation of the Central Balkans comprises 35 plant communities within the two classes *Therosalicornietea* and *Festuco-Puccinellietea* occurring in the Pannonian Plain and central Balkan (Dajić Stevanović *et al.*, 2016; Lukovic, 2019). These communities develop within different types of saline habitats, from saline salt marshes to wet/moderately wet salines following a moisture gradient. The ecologically important and very vulnerable are the annual succulent communities on extremely salt-affected soils from the class *Therosalicornietea* of very limited distribution. Of practical importance is the more spread vegetation of salt meadows and meadow-steppes of the class *Festuco-Puccinellietea*.

A total number of 133 salt tolerant species was observed within the 18 studied phytocoenological relevés. Considering the biological groups (grasses, legumes and other salt tolerant herbs), about 13% of the total species were grasses (e.g., *Agrostis stolonifera, Bromus commutatus, Bromus hordaceus, Cynodon dactylon, Festuca pseudovina, Festuca valesiaca, Hordeum hystrix, Puccinellia limosa*, etc.). Legumes made about 10% of the total flora (e.g., *Lotus corniculatus, Lotus teniufolius, Trifolium fragiferum, Trifolium repens, Trifolium patens*, etc.). The remaining plants occurring in the studied areas (77%) included typical euhalophytes and salt-tolerant plants (e.g., *Artemisia santonicum, genus Atriplex, Camphorosma annua, Camphorosma monspeliaca, Suaeda maritima, Spergularia salina, Plantago schwartzenbergiana, Aster tripolium* ssp. *pannonicus*, etc.) and sporadically some other species (*Lepidum ruderale*, *Cynodon dactilon*, *Inula britanica* etc.). Researched halophytic vegetation mainly belongs to the class *Festuco-Puccinellietea*, represented by two alliances *Puccinellion limosae* (Alliance 1) and *Festucion pseudovinae* (Alliance 2)(Figure 2). These alliances consist of several plant communities such as *Puccinellietum limosae*, *Camphorosmetum annuae, Hordeetum hystricis, Lepidio crassifolii-Puccinellietum limosae, Achilleo-Festucetum pseudovinae, Artemisio santonici-Festucetum pseudovinae*, which were observed in the studied area.

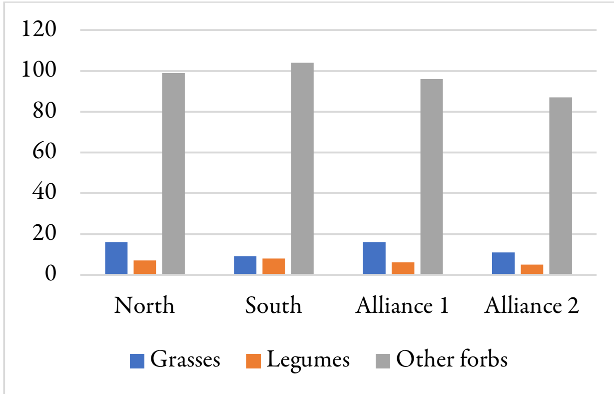

Figure 2. Total number of species in each biological group along the geographical gradient and within halophytic alliances *Puccinellion limosae* (Alliance 1) and *Festucion pseudovinae* (Alliance 2)

Chemical composition of halophytic grasslands

The chemical composition of biomass was determined separately for each of 18 studied halophytic grasslands sites (Table 2). The results were analysed from three aspects: 1. Variability per site; 2. Variability along the geographical gradient; 3. Variability between alliances.

In terms of analysed quality parameters per site, DM value ranged from 92.06% to 95.67%. The maximum value of DM was identified at site L4, which belongs to *Puccinelion limose* alliance with dominance of *Puccinellia limosa*, *Cynodon dactylon* and *Atriplex hastata*, while the minimum value of DM was found at site L2, belonging to the same alliance, but with dominance of the *Puccinellia limosa, Atriplex prostrate* and *Statice gmelini*. Ash content varied from 8.88% at site L16 (all. *Pucinellion limosae*) to 5.64% at site L5 (all. of *Festucion pseudovinae*). The percentages referring to crude protein (CP) ranged from 12.23% for L9 (prevailing of *Agrostis stolonifera, Hordeum hysterics* and *Festuca pratensis*) to 4.42% for site L4. The highest cellulose content (37.59%) corresponded to site L7 (all. *Festucion pseudovinae*), while the lowest (25.8%) was found at site L18 (all. *Puccinellion limosae*). The fat content varied between 3.82% at site L8 (all. *Puccinellion limosae*, dominant species: *Puccinellia limosa*, *Plantago maritima* and *Lepidium cartilagineum*) and 1.04% at site L12 (all. *Puccinellion limosae*, dominant species: *Agrostis stolonifera, Aster tripolium,* and *Puccinellia limosa*). Maximum BEM content (57.49%) was identified at site L6 (all. *Festucion pseudovinae*) and minimum (49.06%) at site L7 (all. *Puccinellion limosae*). The highest NDF content (66.64%) was determined for site L16 (all. *Puccinellion limosae*) and the lowest (52.28%) for site L10 (all. *Festucion pseudovinae*). The highest ADF content (45.09%) was recorded for site L7 (all. Puccinellion limosae, dominant species: *Puccinellia limosa, Camphorosma annua,* and *Cynodon dactylon*) and the lowest (29.31%) for site L18 (all. *Puccinellion limosae*, dominant with *Puccinellia limosa, Aster tripolium,* and *Cynodon dactylon*). Shannon-Wiener diversity index varied from 3.22% for site L13 (all. *Puccinellion limosae*, species *Puccinellia limosa, Cynodon dactylon,* and *Trifolium fragiferum*) to 1.24% for site L4, which belongs to *Puccinellion limose* alliance with dominance of *Puccinellia limosa, Cynodon dactylon,* and *Atriplex hastata*.

In terms of the analysis of quality parameters according to geographical gradient, we found that the average value of CP (9.82%), Ash (7.35%) and BEM (52.17%) were higher for the Central Balkan halophytic grasslands compared to the Pannonian halophytic grasslands with higher values of DM (93.7%), C (30.17%), CF (2.30%).

Comparing two alliances, *Puccinelion limosae* had higher average values for Ash (7.18%), CP (8.66%), C (30.36%), while *Festucion pseudovinae* vegetation had higher values concerning DM (94.72%), CF (2.42%), BEM (54.53%).

Table 2. Chemical composition of sampled plant material (biomass)

Code	DM	Ash	CP	C	CF	BEM	NDF	ADF	Fresh mass (gm^{-2})	Dry mass (gm^{-2})	H
L1	92.22	7.24	80.84	31.95	10.72	50.25	64	39.37	595	149	20.82
L2	92.06	70.7	90.64	23.34	20.01	57.31	57.67	33.55	735	295	20.31
L3	92.08	70.82	10.7	30.09	20.19	49.2	55	39.15	283	164	20.14
L4	95.67	70.03	80.28	26.98	30.03	54.68	58.68	34.64	446	134	10.24
L5	95.45	60.95	11.62	30.3	10.74	49.39	64.89	38.41	142	59	10.71
L6	95.25	60.37	70.23	27.29	10.62	57.49	60.58	36.47	300	107	20.77
L7	92.93	60.51	40.42	37.59	20.42	49.06	54.48	45.09	208	55	10.44
L8	92.65	60.23	90.98	28.95	3.82	51.02	58.79	39.65	353	187	20.81
L9	92.72	60.58	12.23	30.95	10.31	51.32	57.86	40.02	256	87	20.21
L10	93	70.4	80.36	27.48	30.36	53.4	52.28	35.28	464	136	20.04
L11	95.67	70.69	90.07	27.71	2	54.3	59.99	38.26	215	58	2.64
L12	93.2	70.63	80.57	31.44	10.04	51.03	61.25	37.89	261	158	20.03
L13	94.4	60.88	70.6	32.42	20.76	49.98	62.02	40.03	290	167	30.32
L14	95.43	60.45	70.8	27.8	10.97	51.01	63.47	38.72	215	130	10.65
L15	94.94	50.64	60.37	32.37	20.69	52.93	63.82	40.49	317	200	20.1
L16	92.74	80.88	70.11	32.21	20.25	49.55	66.64	38.58	820	342	2.29
L17	92.58	70.66	70.9	29.5	20.18	49.87	62.56	37.1	560	264	10.25
L18	92.61	80.23	90.73	25.8	20.49	53.75	53.28	29.31	993	330	20.15

DM = dry matter (in %); CP = crude protein; CF = crude fat; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; Fresh mass (gm^{-2}); dry mass (gm^{-2}); H=Shannon-Wiener diversity index (%); code (L1-L18) corresponding to sites in table 1; minimum values expressed in yellow; maximum value expressed in turquoise blue

Differences in chemical composition along the geographic gradient

The relationships between halophytic grasslands and the values of the main nutritional parameters were visualized using the DCA ordination diagram (Figure 3). The graph contains passively projected values of the results obtained in the laboratory for each relevé. The results of the chemical analyses were plotted on the DCA ordination diagram.

DCA diagram shows the distribution of each phytocoenological relevé of halophytic grasslands along the 1st and 2nd ordination axes. The relevés distributed along the 1st axis and its relation with quality parameters could be explained by correlation with Ash, BEM and CP for central Balkan vegetation, while pannonian halophytic grasslands related to C, DM and ADF. The relevés along the 2nd axis related to quality parameters correlated to CF and NDF.

The differences between the Pannonian halophytic grassland grouped on the left side and the Central Balkan halophytic grasslands on the right side are evident. Additionally, the Pannonian halophytic grassland could be perceived as two separate groups: 1. Vegetation that mainly develop to moderate wet to dry solonchaks of all. *Puccinellion limosae* and 2. Vegetation of all. *Festucion pseudovinae* on slightly salinized soils, mostly solonetz which is correlated to ash and cellulose content.

The distinction between the Pannonian and the Central Balkan halophytic grasslands is the result of differential species that are typical for north (n.) and south (s.) regions (e.g., n. *Camphorosma annua* - s. *Camphorosma monspeliaca*, n. *Festuca pseudovina* - s. *Festuca valesiaca*, etc.). Although statistical tests did not confirm any significant differences, yet subtle differences do exist. The Central Balkan salt grasslands had higher mean values for CP (7.35%), Ash (9.82%), BEM (52.17%), NDF (60.05%), fresh mass (440.2g), while the Pannonian halophytic grasslands had higher mean values for DM (93.7%), C (30.11%), CF (2.3%), ADF (38.2%), dry mass (170.85g). These results refer to species composition and their abundance within the sampling points. The Shannon-Wiener diversity index showed small differences concerning the Pannonian halophytic grasslands (2.20%) and the Central Balkan (2.04%) ones.

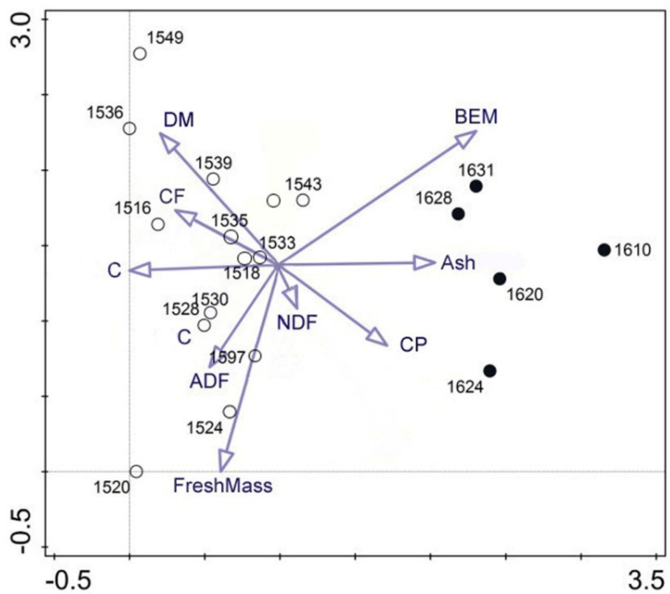

Figure 3. DCA ordination diagram. Results of chemical analysis of plant material (biomass) per each phytocoenological relevé

White circles represent phytocoenological relevés from the north (Pannonian halophytic grasslands); black circles refer to the central-southern of Serbian halophytic grasslands relevés. DM = dry matter (in %); CP = crude protein; CF = crude fat; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; Fresh mass (gm^{-2}); dry mass (gm^{-2})

The main botanical groups (grasses, legumes, other forbs) with selected species based on their abundance in phytocoenological relevés were analysed using Jaccard's similarity index and visually represented using Pyramidal graph to express the differences between the Pannonian and the Central Balkan halophytic grasslands (Figure 4). The results showed that the similarity index is 0.38 for grasses, 0.14 for legumes and 0.15 for other forbs. The absence of some species such as *Festuca pseudovina, Agrostis stolonifera, Bromus hordaceus, Lotus tenuifolius, Camphorosma annua, Aster tripolium* ssp. *pannonicus*, *Salicornia europaea, Suaeda maritima* and etc., in the central Balkan halophytic grasslands is evident, while the Pannonian halophytic grasslands lack in presence of *Bromus commutatus, Festuca valesiaca, Trifolium patens*, and *Camphorosma monspeliaca*.

Comparative analysis of the quality of halophytic alliances

Since the sample size was small, a non-parametric Mann-Whitney U test was performed. The purpose of this test was to determine if there were statistically significant differences in chemical composition between the *Puccinelion limosae* and *Festucion pseudovinae* alliances. The results of the test showed that there were no statistically significant differences in the observed parameters between the compared alliances ($p < 0.05$). However, there were subtle differences in species composition that lead to differences in quality within the studied alliances. The results of the comparative analysis of the main functional groups (grasses, legumes and other forbs) within the *Puccinellion limosae* and *Festucion pseudovinae* alliances showed a similarity index of 0.62 for grasses, 0.43 for legumes and 0.36 for other forbs. The floristic differences were plotted in a Pyramidal graph (Figure 5).

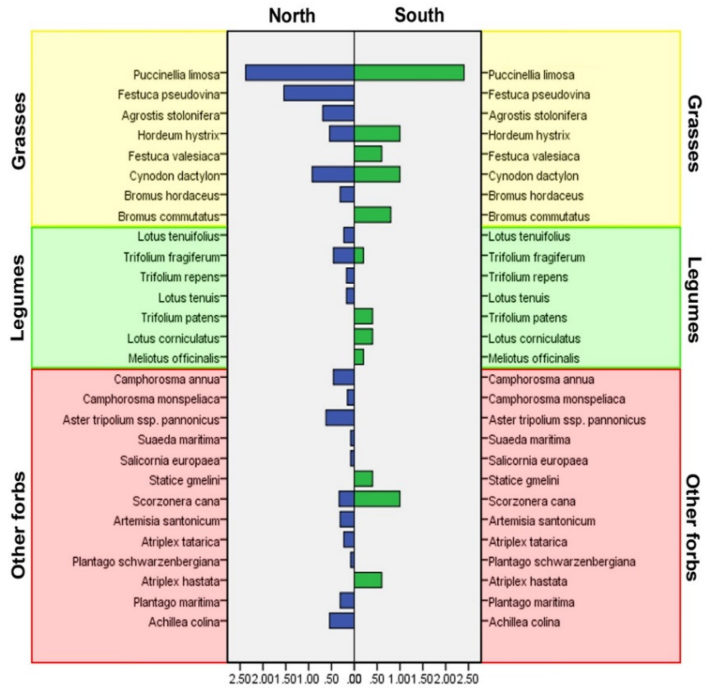

Figure 4. Comparative analysis of botanical group's species composition between the Pannonian and southern halophytic grasslands

Coverage coefficients (scale: r, +, 1-5) were assigned to the list of extracted species present in relevés, where the species with a coefficient greater than 1 were selected;

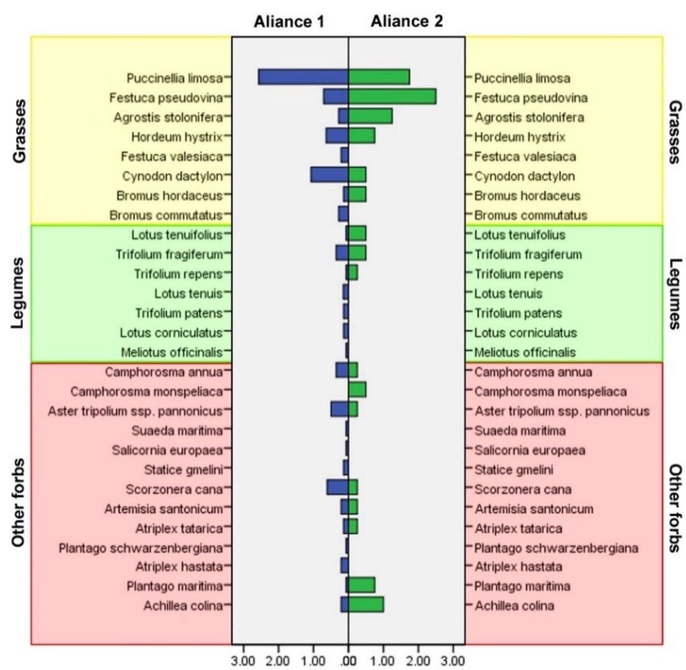

Figure 5. Comparative analysis of botanical groups species composition between studied alliances *Puccinelion limosae* and *Festucion pseudovinae*

Coverage coefficients (scale: r, +, 1-5) were assigned to the list of extrcted species present in relevés, where the species with a coefficient greater than 1 were selected; Alliance 1- *Puccinellion limosae*; Alliance 2- *Festucion pseudovinae*

Discussion

Analysis of the chemical composition of halophytic grasslands compared to quality standards for conventional grasses, legumes and other forbs, show that a mixture of halophytes has an intermediate to low quality ranging from 3 to 5 according to the proposed scale (Marsalis *et al.*, 2009). The results also show that there are no statistically significant differences between the studied halophytic grasslands in terms of geographical distribution from the Pannonian Plain to the Central Balkans, and between the *Puccinellion limosae* and *Festucion pseudovinae* alliances. The minor differences in the chemical composition of halophytic grasslands are the result of floristic diversity and small variations in ecological factors that condition plant communities' patterns.

Quality of halophytic grasslands

Nutritional value: research shows that the quality of halophytic vegetation can vary due to several factors - the chemical composition of the plants themselves, the tissue morphology or their genetic structure; however, the maturity stage of the plant is also very important (Mountousis *et al.*, 2008; Asaadi and Dadkhah 2010; Atasoglu *et al.*, 2010). In addition, external factors are very important, especially climate (rainfall, wind, sunlight, temperature) and soil composition, which can greatly affect the amount of protein and cellulose in plants (Nasrullah *et al.*, 2003). The chemical composition of any animal feed (including halophytes) is the first indicator of its nutritional value. The nutritional value is determined by the concentration of these nutrients (Lalman, 2017). The main quality indicators are the total amount of protein, fat and cellulose. Our results show that there is no statistically significant difference in the chemical composition of the quality parameters among the studied sites, but there are subtle variations in chemical composition caused by species composition in conjunction with ecological conditions (soil properties, moisture content and climate). Other studies reported that there are differences in nutrient levels depending precisely on the maturity stage of the particular studied plant (Ahmadi *et al.*, 2013). Accordingly, this study was conducted at the same vegetation stage and the results obtained are not dependent on the maturity stage but rather on other external factors. Moreover, the crude protein content of legumes is higher than that of grasses (Minson, 1990), while the crude protein content of forbs is intermediate, between the values of legumes and grasses (Cook, 1972; Krysl *et al.*, 1984; Meyer and Brown, 1985; Ruyle, 1993).

The crude proteins: The crude protein content varied from 4.42% to 12.23%, which is comparable with the previous results of other studies on halophytic flora e.g., Tawfik *et al.*, 2015; Attia-Ismail, 2018, etc.). The biomass samples from the locality L8-Trešnjevac, which had the highest value in crude protein content, consist of species that had very high values in protein content in individual analyses in other regions of the world: *Atriplex nummularia* was appointed by 13.68% of the total protein content (Tawfik *et al.*, 2015) to 20.69% in *Atriplex repanda* (Attia-Ismail, 2015). *Atriplex* species have good protein content (Van Niekerk *et al.*, 2004) and sites with dominance of this genus could be suitable for grazing. Regarding the species *Bothrichloa ischaemum* the protein content ranged between 14% in the leaves of this plant to 10.8% in the stems, while the species *Bothrichloa bladhii* has a crude protein value of 14.77% in the vegetative stage, which decreased with the age of the plant to 8.31% (Villanueva-Avalos, 2008). Species of the genus *Lotus*, which also confine to this community, have very high values of crude protein - 14.86. The species *Artemisia monosperm* was characterized by 12.98% of the crude proteins (Tawfik *et al.*, 2015), whereas *Camphorosma monspeliaca* varied in crude protein content from 5.39% in the flowering phase to 11.43% in the early vegetative phase (Ahmadi *et al.,* 2013). The values of crude proteins in the genus *Scorzonera* varied from 6.63% (*S. suberosa*) to 10.05% in *S. temmentos* depending on the species (Tuncturk *et al.,* 2015). The high values of crude proteins (11.62%) at site L5-Suva česma were also attributed to species composition, since it was showed that some have high crude protein values, as estimated for (*Lotus* ssp.: 14.86%, and *Carex* ssp.:15% (Catling *et al.*, 1994), *Cynodon dactylon*: 8.91% (Munir *et al.*, 2020)). According to studies of Abou El Nasr *et al.* (1996) and Badri and Ludidi (2020), where nutritional value of succulent plant parts in fresh or air-dried form was analysed, the silage is the most nutritious feed that can meet the energy and protein requirements of animals.

Cellulose: high levels of cellulose were present in the biomass from localities L7-Žabalj 2 (37.59) and L13-Ruski Krstur (32.42), which included the prevalence of the following species - *Cynodon dactylon, Puccinellia distans, Camphorosma annua, Scorzonera cana,* and *Plantago tenuiflora* for the area of Žabalj, and *Cynodon dactylon, Luzula campestris, Puccinellia distans, Polygonum aviculare, Festuca ovina,* and *Trifolium fragiferum* for L13-Ruski Krstur. The obtained values for cellulose are significantly higher than the data found in the literature for individual species that are part of the biomass of these sites (e.g., for *Cynodon dactylon*: 25.87%) (Munir *et al.*, 2020). If we compare our results with the results obtained for non-saline productive grasslands of the central Balkan, it could be seen that pastures of the community *Festucetum vallesiaceae* had an uniform protein content ranging from 7.60 to 9.53% and cellulose values ranging from 27.49 to 29.55% (Tomić *et al.*, 2009). Even though, in the central Balkan study region there was no species such as *Agrostis stolonifera*, for example, as a carrier of good quality, the studied halophytic grasslands could be considered to provide the biomass of good forage value.

The crude fat: Total fat comprises a group of nutritious fats that play a very important role in the animal body (Liamadis, 2000) and are a very important energy component in animal feed (Bauman *et al.*, 2003). The amount of fat in biomass is usually low (less than 3% of dry matter) (Coleman and Henry, 2002; Bruinenberg, 2003; Koutsoukis *et al.*, 2016). The lowest values for fat in biomass samples were obtained for the plant material from the area of L12-Bački Brestovac (1.04), which contains the species *Agrostis stolonifera, Puccinellia distans, Luzula campestris, Aster tripolium* ssp. *pannonicus, Festuca pseudovina, Lotus tenuis, Cynodon dactylon,* and *Camphorosma annua*, while the highest value for fat was found in plant material from L8-Trešnjevac (3.82). However, the obtained average values were about 3%, which agrees very well with the results of other authors (Kandrelis, 2016). The factors that affect feed quality include environmental factors, palatability, nutrient content (chemical composition), presence of different secondary metabolites, feeding value (voluntary intake by animals, digestibility of nutrients) and finally animal performance (Attia-Ismail, 2018). In saline habitats the halophytes have the advantage because of salinity tolerance (Ahmadi *et al.*, 2013) and are highly competitive with non-halophytes.

The analysis of the main quality parameters was used to determine biomass quality (crude protein content ranged from 12.23% to 4.42%, cellulose content from 37.59% to 23.34% and fat content from 3.82% to 1.04%) indicating that South-east European halophytic vegetation is acceptable for animal feeding. The studied sites with generally higher cellulose values belong to the slightly salinized solonetz with dominance of grasses, while the heavily salinized solochaks are typical for dominance of euhalophytes exhibiting higher values of protein content. As showed in previous studies, the pastoral values vary along the soil salinity gradient (Dajic-Sevanovic *et al.*, 2008). According to Masters (2015), halophytic vegetation has some limitations in animal feeding, mainly due to palatability, and less due to to the chemical composition. Field observations indicated that vegetation of salt-affected soils, ranging from slightly saline to severely saline, is under grazing or mowing management. Some species of saline grasslands have moderate to high nutrition quality (e.g. genus *Lotus, Atriplex, Meliolotus*, etc. (Marinoni *et al.*, 2019), which points out the possible use of saline grasslands as fodder source.

Halophytic plants occur in different saline habitats upon specific environmental conditions, season, and geographic region, and vary significantly in their chemical composition and nutritional value (Attia-Ismail, 2018). They are characterised by complexity and polymorphism of saline habitats (Grigore *et al.*, 2014). Ecological, biogeographical and climate conditions, contributed to the certain differences in biomass quality between the studied Pannonian and central Balkan saline grasslands based, due to differences in species composition. Central Balkan saline grasslands are distributed in areas of drier climate and sub-Mediterranean climatic influence, where several low-protein grass species dominated (e.g., *Puccinellia festuciformis* ssp. *convoluta*, *Puccinellia limosa*, *Bromus commutatus etc.*). On the contrary, wet/moderately wet saline grasslands in Pannonia tend to be dominated by cellulose- and fat-rich grasses (*Agrostis stolonifera, Puccinellia* *limosa, Fesuca pseudovina*). According to Dajic-Stevanovic *et al.*, (2008), halophytic communities with salt-tolerant plants such as *Achillea millefolium* subsp. *collina*, *Trifolium filiforme* and *Agropyrum repens* are classified as very good according to pastoral value, while biomass with dominance of *Lotus tenuis* or *Trifolium fragiferum* is excellent. The lowest nutritional value has a group of species belonging to the salt tolerant species (e.g., *Suaeda maritima, Salicornia europaea* and some other *Amranthaceae*.

The main reason for the low variation in pastoral quality between the studied regions is the fact that saline grasslands are usually composed of small number of species, where only one or few of them highly dominate, (Landucci *et al.*, 2015) while most of other plants of floristic spectra are designated with abundance less than 5%. Species that are carriers of high fodder quality could not be detected. The second characteristic of halophytic grasslands is the rapid spatial change/shift of plant communities in a small area due to variations in ecological conditions, especially in the soil salt content as the key determinant (Luković *et al.*, 2020). Small differences in salt content and the soil moisture frequently result in occurrence of specific vegetation units distributed on very small patches (average 1 m^2 to 16 m^2), with few or even only one dominant species (Stevanović *et al.*, 2019). Furthermore, the high abundance of some grasses of respectable forage quality, such as *Puccinellia limosa, Festuca pseudovina or Festuca valesiaca, Agrostis stolonifera* points out the significance of halophytic grasslands for animal feeding. Moreover, some other herbs of salt affected soils, such as *Artemisia santonicum, Statice gmelini, Camphorosma annua, Camphorosma monspeliaca*, which account for the main amount of biomass, followed by legume species from the genus *Trifolium* or *Lotus* with small abundance contributed to the overall quality value of halophytic grasslands which is therefore not exceptional.

Finally, continental halophytic grasslands showed satisfied quality, even though did not express differences in quality along the geographic gradient, as well as between studied alliances. With intention to explain lack of differences and factors that contributing to, we highlighted floristic patterns and complex nature of halophytic vegetation as important factor.

Conclusions

The Pannonian Plain is the largest area of saline soils and continental halophytic vegetation in the Europe. In addition, there are sporadic patches of continental saline habitats in the south Balkans, and due to geographic and climatic impacts, obvious differences in floristic composition and vegetation types exist. In the studied region, different environmental conditions provide a wide range of halophytic flora and vegetation occurring on different types of salt affected soils, including highly salinized solonchaks to solonetz soil types, Halophytes may play a role in "saline agriculture" as animal feed or human food, energy crops for fuel production, oils, pharmaceuticals, etc., but are an important source of animal feed in this and similar regions. The chemical values of the main nutrients of the halophytic biomass vary depending on the floristic composition. The results show that there are no statistically significant differences in the quality of chemical composition between two distinct study sites, but only a small range of variation due to differences in floristic composition. The possible relations between salt-affected soil type, floristic patterns, chemical analysis and suitability for grazing/mowing or management of halophytic grasslands could be a challenge for further studies.

Authors' Contributions

Conceptualization: ML, UŠ, JV, JR, GT, MK, ZDS. Field work and collection of samples: ML, JV, MK; chemical analysis: JR; statistical analysis: UŠ; GIS data preparing and editing text: GT; conceived the idea and designed the work and supervision: ZDS.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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