Received: 15 May 2019 | Revised: 13 October 2019 | Accepted: 14 November 2019

DOI: 10.1002/leg3.23

ORIGINAL RESEARCH ARTICLE

Legume Science Open Access WILEY

The variation of the chemical composition of the main plant species in a subalpine grassland in northwestern Greece

Charalampos Koutsoukis[1] | Christos Roukos[1] | Panagiotis G. Demertzis[2] | Sotirios Kandrelis[1] | Konstantoula Akrida-Demertzi[2]

[1]Department of Agriculture, University of Ioannina, Arta, Greece

[2]Department of Chemistry, Section of Industrial and Food Chemistry, Food Chemistry Lab., University of Ioannina, Ioannina, Greece

Correspondence
Konstantoula Akrida-Demertzi, Department of Chemistry, Section of Industrial and Food Chemistry, Food Chemistry Lab., University of Ioannina, PO Box 1186, Ioannina GR 45110, Greece.
Email: kakrida@uoi.gr
Charalampos Koutsoukis, Department of Agriculture, University of Ioannina, Arta 47100, Greece.
Email: koutsoukis.har@hotmail.com

Present address:
Christos Roukos, Ministry of Rural Development and Food, Regional Department of Epirus & Western Greece, Terma Panepistimiou Str, Ioannina 45110, Greece.

Abstract

This study presents the results of a survey conducted in the year 2013 in a subalpine grassland located in Epirus, in northwestern Greece, and concerns the determination of the chemical composition of nine wild plants species (*Alopecurus gerardii* V., *Stipa pennata* L., *Phleum alpinum* L., *Trifolium repens* L., *Lathyrus aphaca* L., *Lotus corniculatus* L., *Ranunculus repens* L., *Achillea millefolium* L., and *Geranium lucidum* L.) at various stages of growth. The highest mean neutral detergent fiber content was determined in *S. pennata* L. samples with 546 g kg^{-1} dry matter (DM). The highest mean acid detergent fiber content was determined in samples of *A. gerardii* L. with 358 g kg^{-1} DM. For calcium (Ca), the highest average content was determined in the samples of *L. corniculatus* L. with 9.6 g kg^{-1} DM. For phosphorus (P), iron (Fe), and copper (Cu), the highest average contents were determined in the samples of *R. repens* L. with 2.8 g kg^{-1} DM, 60.2 mg kg^{-1}, and 5.6 mg kg^{-1} DM, respectively. For magnesium (Mg), the higher average content was found in *L. aphaca* L. samples with 1.6 g kg^{-1}, for potassium (K) in *G. lucidum* L. samples with 15.6 g kg^{-1}, for sodium (Na) in *A. millefolium* L. samples with 15.3 g kg^{-1}, and for manganese (Mn) in *P. alpinum* L. samples with 37.9 mg kg^{-1}. The chemical composition of plant species meets the nutritional requirements of grazing animals in the investigated area. Among the plant species, legumes and other forbs have the highest nutritional value.

KEYWORDS

chemical composition, subalpine grassland, wild plants

1 | INTRODUCTION

In the Mediterranean countries, grasslands constitute a very important terrestrial resource (Cosentino et al., 2014). In the countries of the European Union, they occupy an area of 56 million ha, of which 17.5 millions are mainly located in the mountainous areas (Peeters, 2008; Peyraud & Peeters, 2016). In Greece, grasslands occupy an area of about 1.67 million ha, of which 400,000 ha are mainly subalpine grasslands (Vrachnakis, 2015). Ruminant animals cover a large part of their dietary needs by grazing (Zervas, 1998). Grazing-based rearing systems give animal products of high nutritional value (Buchin, Martin, Dupont, Bornard, & Achilleos, 1999; Noziere et al., 2006; Viallon et al., 2000) characterized by their authenticity, quality, and originality (Chatzitheodoridis, Michailidis, & Theodossiou, 2007; McMorran et al., 2015). The rational management of grazing contributes to the preservation and protection of each ecosystem (Rook et al., 2004).

The nutritional value of rangeland plants depends on their chemical composition. Their main components are proteins and crude fiber.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2019 The Authors. *Legume Science* published by Wiley Periodicals, Inc.

Generally, the higher the crude fiber content of the plants, the lower their nutritional value for the animals (Papanastasis & Ispikoudis, 2013).

Plants differ in their chemical composition. These differences are evident both between plants and during their various stages of growth (Elgersma & Søegaard, 2016). The same plant species at the same stage of growth differ significantly in their chemical composition when grown in different environments, even when in nearby environments (Belkhir et al., 2013; Buxton, 1996; Tamburino et al., 2012).

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) are important indicators for determining the nutritional value of rangeland plants, as they have a significantly negative relationship with digestibility (Buxton & Redfearn, 1997; Earle, Kienzle, Opitz, Smith, & Maskell, 1998; Elgersma & Søegaard, 2016). NDF and ADF content of plants increases as plants mature (Moreira, Prado, Cecato, Wada, & Mizubuti, 2004; Mountousis, Papanikolaou, Stanogias, Chatzitheodoridis, & Roukos, 2008a). The stems and shoots of the rangeland plants contain higher levels of crude fiber than leaves. The increase in crude fiber content of plants as the plants grow is due to a decrease in the leaf/stem ratio (Buxton & Redfearn, 1997). NDF and ADF contents are generally higher in grasses than in legumes and other forbs (Elgersma & Søegaard, 2016; Foroughbakhch, Hernandez-Pinero, & Carrillo-Parra, 2012; McCollum, Galyean, Krysl, & Wallace, 1985; Tufarelli, Cazzato, Ficco, & Laudadio, 2010a).

When the mineral content of the grassland is low, the quality of the grassland production is affected as animal feed intake decreases, thereby adversely affecting microbial growth and activity of the large abdomen. This leads to a decrease in the digestibility of forage (Leng, 1990; Provenza, 1995). The forage content in minerals depends on many factors such as soil, climate, season of the year, plant species and plant growth, and fertilization (Flisch, Sinaj, Charles, & Richner, 2009; Georgievskki, Annenkov, & Samokhin, 1982; Öborn et al., 2008; Ramírez-Pérez, Buntinx, & Rosiles, 2000).

The leaves are richer in minerals than the stems (Myrvang, Hillersøy, Heim, Bleken, & Gjengedal, 2016; Tan, Bakoglu, & Koc, 1997). Grasses, in comparison with legumes and other forbs, have generally lower macroelement content, with the exception of sodium (Na). Other forbs usually have higher phosphorus (P) and potassium (K) content than legumes but lower magnesium (Mg) and calcium (Ca) content, while also having lower sodium (Na) content than grasses and legumes (Elliot, 1986; McDowell, 2003).

In grasslands, P is usually the most inadequate element. Its lack in livestock feed has resulted in reduced livestock reproduction (Hale & Olson, 2001; McDowell, Ellis, & Conrad, 1984; McDowell & Valle, 2000). In order to meet the dietary needs of ruminants, the rangeland plants should contain at least 3 g kg^{-1} Ca, 2 g kg^{-1} Mg, and 8 g kg^{-1} K (Tajeda, Mcdowell, Martin, & Conrad, 1985). According to Ward and Lardy (2005), the forage has a relatively high K content ranging from 10 to 20 g kg^{-1} dry matter (DM). Also, the copper (Cu) content of rangeland plants adequately meets the needs of most livestock (Corah & Dargatz, 1996; Greene, 2000; Greene, Baker, & Hardt, 1989).

The mineral content of plants varies depending on their stage of growth. In general, the mineral content of plants decreases as the plants mature (Pirhofer-Walzl et al., 2011; Schlegel, Wyss, Arrigo, & Hess, 2016). Tan, Temel, and Yolcu (2003) reported that the K, Mg, Ca, and P content of plants decreased from 29.31 to 22.04, 3.48 to 2.85, 12.83 to 11.58, and 1.50 to 1.19 g kg^{-1}, respectively, as the plants matured.

Sheep needs in Ca range from 2 to 8.2 g kg^{-1} DM, in P, the minimum requirements are 1.6 g kg^{-1} DM, in Mg range from 1.2 to 1.8 g kg^{-1} DM, in K from 5 to 8 g kg^{-1} DM, in Na from 0.9 to 1.8 g kg^{-1} of DM, in Fe, the minimum requirements are 30 mg kg^{-1} DM, in Mn are 20 mg kg^{-1} DM, and in Cu range from 20 to 30 mg kg^{-1} DM (National Research Council, 1985). According to Zervas (2013), the daily maintenance requirements of a 50-kg sheep are 5 g day^{-1} in Ca, 0.7 g day^{-1} in Mg, 3.5 g day^{-1} in P, and 1.25 g day^{-1} in Na, whereas the minimum requirements in Mn and Cu are 30 and 5 ppm day^{-1}, respectively.

The aim of the present study was to determine the variation in the chemical composition of the main plants of the subalpine grassland of "Kostilata" by botanical group, as their chemical composition determines the nutritional value of the forage. The knowledge of the nutritional value of plants will contribute to the rational management of grazing as well as to the preservation and protection of the ecosystem.

2 | MATERIAL AND METHODS

2.1 | Study area

The study was conducted in 2013, in "Kostilata" subalpine grassland. This area extends at an altitude of 1,100 to 2,393 m and it is located in Theodoriana, Epirus, Western Greece. It occupies an area of 950 ha with 3,500 sheep grazing during the summer months.

In "Kostilata" grassland, there are 96 taxa. Within the grassland itself, there is a variation in the physicochemical properties of the soil and its inclinations, depending on the altitude. At an altitude of 1,100 to 1,400 m, the soil contents of sand, silt, clay, organic matter, available P, and pH value were found at 43.9%, 38.3%, 17.8%, 6.9%, 4.2 mg kg^{-1}, and 5.9, respectively. At an altitude of 1,401 to 1,800 m, the soil contents of sand, silt, clay, organic matter, available P, and pH value were found at 48.7%, 35.1%, 16.2%, 6.7%, 5.6 mg kg^{-1}, and 5.5, respectively. At an altitude of 1,801 to 2,393 m, the soil contents of sand, silt, clay, organic matter, available P, and pH value were found at 52.9%, 33.9%, 13.2%, 5.5%, 6.2 mg kg^{-1}, and 5.4, respectively. Most of the mild slopes (from 0% to 30%) are at an altitude of 1,100 to 1,400 m, whereas most of the steep slopes (over 60%) are at altitudes above 1,800 m (Roukos et al., 2016).

2.2 | Plant samplings analyses

Sixty (60) fixed experimental cages of 4 m × 4 m in size were installed and enclosed by a fence of 1 m high, in order to protect the plants

from grazing. The cages were placed randomly and in such a way so as to be representative of the grassland. Samples were collected approximately every 10 days, from late April to mid-July. Three plant species were selected from each plant group (grasses, legumes, and other forbs) that contained the highest percentage of biomass. Specifically, *Alopecurus gerardii* V., *Stipa pennata* L., and *Phleum alpinum* L. species were selected from grasses, *Trifolium repens* L., *Lathyrus aphaca* L., and *Lotus corniculatus* L. species from legumes, and *Ranunculus repens* L., *Achillea millefolium* L., and *Geranium lucidum* L. species from other forbs.

For the identification of the plant species, the encyclopedia "Mountain Flora of Greece I and II" (Strid, 1986; Strid & Tan, 1991), the book "The main grasses of natural grasslands" (Papanastasis & Karagiannakidou - Papadimitriou K., 1993), and the book "Vascular Plants of Greece" (Dimopoulos et al., 2013) were used. The samples were placed in an oven for drying at 65°C for 48 hr (Deinum & Maassen, 1994) and milled to particle size of 1 mm using a Kinematica Polymyx PX-MFC 90 D cutting mill.

The NDF content was determined according to the method of Van Soest, Robertson, and Lewis (1991) as modified by Vogel, Pedersen, Masterson, and Toy (1999) using an ANKOM 2000 fiber analyzer. The samples were analyzed using heat stable amylase without sodium sulfite in the neutral detergent reagent. The ADF content was determined according to AOAC (1997, method 973.18). NDF and ADF were expressed without residual ash. P was determined spectrophotometrically using a HITACHI, U-1800 spectrophotometer. Ca, Mg, K, Na, Cu, Fe, and Mn were determined by atomic absorption spectrometry using a PERKIN ELMER atomic absorption spectrometry instrument (AOAC, 1999, method 968.08).

A weather station that was already installed at an altitude of 1,100 m was used to obtain the climatic parameters (air temperature and precipitation). For the same purpose, two new weather stations were installed, in spring 2013, at an altitude of 1,600 and 2,050 m, respectively.

2.3 | Statistical analysis

The results were compared for significant differences by one-way analysis of variance test, whereas mean differences were checked using Tuckey's test ($P < .05$). Statistical analyses were performed with OriginPro 9.0 software.

3 | RESULTS AND DISCUSSION

3.1 | Precipitation and temperature

Different climatic conditions prevail within the "Kostilata" grassland. Higher temperatures at lower altitudes were recorded throughout the investigation. In May, higher precipitation was observed at lower altitudes, whereas in June and July at higher altitudes (Table 1). The last 10 days of April, only *R. repens* L. species was found in the grassland, whereas *P. alpinum* L. species was found for the first time in the first 10 days of June.

3.2 | NDF and ADF content

The highest mean NDF content was found in the samples of *S. pennata* L. species with 546 g kg^{-1} DM, with a statistically significant difference ($P < .05$) from the other plant species except *A. gerardii* V. (Table 2). The highest mean ADF content was found in the samples of *A. gerardii* V. species with 358 g kg^{-1} DM, with statistically significant difference ($P < .05$) from other plant species, except *S. pennata* L. and *A. millefolium* L. (Table 3). In all plant species, the lowest NDF and ADF contents were determined in the samples of the first samplings and then a gradual increase in NDF and ADF values was observed, with statistically significant differences ($P < .05$). Also, statistically significant differences ($P < .05$) were observed between the values of different plant species at the same sampling dates (Tables 2 and 3).

3.3 | Macrominerals

Concerning the average mineral content of plant species, a significant variation was observed. The highest mean Ca content occurred in the samples of *L. corniculatus* L. species with 9.6 g kg^{-1} DM, with statistically significant differences ($P < .05$) observed only with the mean values of grasses. Between sampling times, statistically significant differences ($P < .05$) were observed only in grass samples (Table 4).

The highest mean P content was found in *R. repens* L. species with 2.8 g kg^{-1} DM, with statistically significant differences ($P < .05$) observed only with the mean values of legumes and *S. pennata* L. species. Statistically significant differences between sampling periods were observed only in *T. repens* L. and *R. repens* L. species (Table 5).

The highest average Mg content was found in *L. aphaca* L. species with 1.6 g kg^{-1} DM. Statistically significant differences ($P < .05$) were observed between the mean values of *L. aphaca* L. and the mean values of grasses and *G. lucidum* L. species (Table 6). For K, the highest mean content was found in the samples of *G. lucidum* L. species with 15.6 g kg^{-1} DM, without any statistically significant differences ($P < .05$) with the mean values of other plants. Statistically significant differences between sampling periods were observed in *A. gerardii* V., *S. pennata* L., *L. aphaca* L., *R. repens* L., *A. millefolium* L., and *G. lucidum* L. species (Table 7).

For Na, the highest average content was found in *A. millefolium* L. species with 15.3 g kg^{-1} DM, with statistically significant difference observed with mean values of grasses and *L. corniculatus* L. species. Among the sampling periods, no statistically significant differences were observed for *T. repens* L. and *L. aphaca* L. species only (Table 8).

TABLE 1 Precipitation and air temperature during the study period on the "Kostilata" grassland

	Mean precipitation (mm)				Mean air temperature (°C)			
Altitude (a.s.l.) (m)	May	June	July	Aug.	May	June	July	Aug.
1,100	176	59	36	4	13.4	20	23	21.3
1,600	93.4	72	66.4	7	12.5	14.8	17.1	18.5
2,050	110	65	63	4	9.2	10.1	12.9	14.4

TABLE 2 Neutral detergent fiber content of plant species, at various stages of growth (g kg^{-1} dry matter)

		Sampling dates							
Group	Species	April 30	May 16	May 27	June 8	June 17	June 28	July 15	Mean
Grasses	*A. gerardii*	—	436a^{1}	447a^{1}	466a^{1}	550b^{1}	610c^{1}	693d^{1}	534^{1}
	S. pennata	—	417a^{1}	454b^{1}	494c^{1}	571d^{1}	653d^{2}	687d^{1}	546^{1}
	P. alpinum	—	—	—	422a^{2}	465b^{23}	505c^{3}	601d^{2}	498^{2}
Legumes	*T. repens*	—	332a^{2}	364a^{2}	419b^{2}	455bc^{2}	484c^{3}	521d^{3}	429^{3}
	L. aphaca	—	327a^{2}	383b^{2}	409bc^{2,3}	431c^{2}	484d^{3}	563e^{2,3}	433^{3}
	L. corniculatus	—	307a^{2}	383b^{2}	415c^{2}	440c^{2}	505d^{3}	564e^{2,3}	436^{3}
Other forbs	*R. repens*	303a	359b^{3}	413c^{3}	451cd^{1}	480d^{3}	530e^{4}	—	423^{3}
	A. millefolium	—	315a^{2}	365b^{2}	432c^{2}	476d^{3}	551d^{4}	581d^{2}	453^{3}
	G. lucidum	—	302a^{2}	351b^{2}	389b^{3}	445c^{2,3}	485c^{3}	549d^{3}	420^{3}

Note. Mean values followed by different letters (a, b, c, d, and e), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ($^{1, 2, 3,}$ and ^{4}), in the same column, differ significantly ($P < .05$).

TABLE 3 Acid detergent fiber content of plant species, at various stages of growth (g kg^{-1} dry matter)

		Sampling dates							
Group	Species	April 30	May 16	May 27	June 8	June 17	June 28	July 15	Mean
Grasses	*A. gerardii*	—	261a^{1}	292b^{1}	329b^{1}	369c^{1}	430d^{1}	461d^{1}	358^{1}
	S. pennata	—	270a^{1}	304b^{1}	326c^{1}	354c^{1}	426d^{1}	451d^{1}	355^{1}
	P. alpinum	—	—	—	266a^{2}	325b^{2}	339b^{2}	375c^{2}	326^{2}
Legumes	*T. repens*	—	213a^{2}	272b^{2}	314c^{1,4}	372d^{1}	378d^{3}	386d^{2}	322^{2}
	L. aphaca	—	200a^{2}	281b^{1,2}	336c^{1,3}	361cd^{1}	393de^{3}	4204e^{3}	332^{2}
	L. corniculatus	—	199a^{2}	300^{1,2}	350c^{1,3}	378d^{1}	392de^{3}	417e^{3}	339^{2}
Other forbs	*R. repens*	195a	241b^{3}	294c^{1,2}	338d^{1,3}	367e^{1}	371e^{3}	—	301^{3}
	A. millefolium	—	217a^{2}	330b^{3}	346bc^{3}	372c^{1}	405d^{4}	416d^{3}	348^{1,2}
	G. lucidum	—	207a^{2}	242b^{4}	295c^{4}	349d^{1,2}	393e^{3,4}	406e^{2,3}	315^{2,3}

Note. Mean values followed by different letters (a, b, c, d, and e), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ($^{1, 2, 3,}$ and ^{4}), in the same column, differ significantly ($P < .05$).

3.4 | Microminerals

The highest average Fe content was found in *R. repens* L. species with 60.2 mg kg^{-1} DM, with statistically significant differences observed only between the above value and the mean values of *S. pennata* L., *P. alpinum* L., and *G. lucidum* L. species. Statistically significant differences between sampling periods were observed in *T. repens* L., *L. corniculatus* L., and *A. millefolium* L. species (Table 9).

The highest average Mn content was observed in *P. alpinum* L. species with 37.9 mg kg^{-1} DM, with statistically significant differences ($P < .05$) observed between the mean values of legumes and *G. lucidum* L species. Statistically significant differences between sampling periods were observed in *A. gerardii* V. and *A. millefolium* L. species (Table 10).

Concerning Cu, the highest average content was found in *R. repens* L. species with 5.6 mg kg^{-1} DM, with statistically significant differences ($P < .05$) observed in all other plant species, except *L. aphaca* L. and *A. millefolium* L. Statistically significant differences between sampling periods were observed in all plant species, except *L. aphaca* L., *L. corniculatus* L., and *A. millefolium* L. (Table 11).

TABLE 4 Calcium (Ca) content of plant species, at various stages of growth (g kg^{-1} dry matter)

		Sampling dates							
Group	Species	April 30	May 16	May 27	June 8	June 17	June 28	July 15	Mean
Grasses	*A. gerardii*	–	4.8a[1]	2.5b[1]	1.6c[1]	1.5bc[1]	4.0a[1]	1.5bc[1]	2.7[1]
	S. pennata	–	7.0a[2]	1.6b[2]	1.8b[1]	2.3b[1]	1.2b[2]	1.5b[1]	2.9[1,3]
	P. alpinum	–	–	–	1.0a[1]	1.4a[1]	2.1b[3]	1.4a[1]	1.6[1]
Legumes	*T. repens*	–	7.5a[1]	8.9a[2]	9.9a[2]	8.6a[2,3,4]	8.6a[1]	9.4a[1]	9.1[2]
	L. aphaca	–	7.3a[1]	7.0a[1]	10.0a[2]	10.4a[3,4]	7.4a[1]	12.3a[1]	9.4[2]
	L. corniculatus	–	8.9a[1]	10.5a[2]	10.9a[2]	10.0a[4]	8.2a[1]	8.7a[1]	9.6[2]
Other forbs	*R. repens*	5.5a	5.3a[1]	5.9a[1,3,4]	5.8a[1,2]	9.6a[2,3,4]	9.0a[1]	–	6.8[2,3]
	A. millefolium	–	4.3a[1]	7.3a[2,3]	6.9a[1,2]	7.5a[1,4]	12.5a[1]	5.2a[1]	7.6[2]
	G. lucidum	–	11.1a[1]	8.9a[2,4]	6.9a[1,2]	7.5a[1,4]	6.2a[1]	6.0a[1]	7.8[2]

Note. Mean values followed by different letters (a, b, and c), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ([1, 2, 3, and 4]), in the same column, differ significantly ($P < .05$).

TABLE 5 Phosphorus (P) content of plant species, at various stages of growth (g kg^{-1} dry matter)

		Sampling dates							
Group	Species	April 30	May 16	May 27	June 8	June 17	June 28	July 15	Mean
Grasses	*A. gerardii*	–	2.3a[1,2]	2.4a[1]	1.7a[1]	1.6a[1]	1.9a[1]	2.2a[1]	2.0[1,2]
	S. pennata	–	1.1a[1]	1.4a,b[1]	2.1a,b[1]	2.6b[1]	1.8a,b[1]	1.2a,b[1]	1.7[1,3,4]
	P. alpinum	–	–	–	2.2a[1]	1.6a[1]	2.0a[1]	2.0a[1]	1.9[1,2]
Legumes	*T. repens*	–	1.6a[1]	1.7a[1]	1.6a[1]	1.7a[1]	1.5a[1]	0.7a[1]	1.5[1]
	L. aphaca	–	1.4a[1]	1.8a[1]	1.5a[1]	0.9[1]	1.9[1]	0.5a[1]	1.3[1]
	L. corniculatus	–	0.7a[1]	1.7a[1]	2.4a[1]	1.4a[1]	0.2a[1]	1.4a[1]	1.5[1,3]
Other forbs	*R. repens*	4.9a	3.1a,b[2,3]	3.1a,b[1]	1.8b[1]	2.4a,b[1]	2.1a,b[1]	–	2.8[2]
	A. millefolium	–	2.9a[2,3]	2.6a[1]	2.9a[1]	2.3a[1]	2.4a[1]	2.2a[1]	2.5[2,3]
	G. lucidum	–	4.4a[3]	2.6a[1]	2.5a[1]	2.7a[1]	2.4a[1]	2.1a[1]	2.7[2,4]

Note. Mean values followed by different letters (a and b), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ([1, 2, 3, and 4]), in the same column, differ significantly ($P < .05$).

TABLE 6 Magnesium (Mg) content of plant species, at various stages of growth (g kg^{-1} dry matter)

		Sampling dates							
Group	Species	April 30	May 16	May 27	June 8	June 17	June 28	July 15	Mean
Grasses	*A. gerardii*	–	0.6a[1]	0.6a[1,2]	0.7a[1]	0.6a[1]	0.8a[1]	0.7a[1]	0.7[1]
	S. pennata	–	1.2a[1]	0.5a[1]	0.7a[1]	0.8a[1,2]	0.7a[1]	0.6a[1]	0.8[1,4]
	P. alpinum	–	–	–	07a[1]	0.9a[1]	1.2a[1]	1.2a[1]	1.0[1,2]
Legumes	*T. repens*	–	1.4a,b[1]	1.0b[2,3]	1.3a,b[1]	1.2a,b[1,2]	1.1a,b[1]	1.8a[1]	1.3[2,3]
	L. aphaca	–	1.5a[1]	1.2a[3]	1.2a[1]	2.0a[2]	0.8a[1]	2.4a[1]	1.6[3]
	L. corniculatus	–	1.2a[1]	1.2a[3]	1.3a[1]	1.2a[1,2]	1.5a[1]	1.3a[1]	1.3[2,3,4]
Other forbs	*R. repens*	1.3a	0.8a[1]	0.9a[1,3]	1.1a[1]	1.6a[1,2]	1.5a[1]	–	1.2[1,2,3]
	A. millefolium	–	1.5a[1]	1.2a[3]	1.1a[1]	1.2a[1,2]	1.3a[1]	1.3a[1]	1.3[2,3,4]
	G. lucidum	–	0.9a[1]	1.0a[1,3]	1.0a[1]	1.1a[1,2]	1.0a[1]	0.8a[1]	1.0[1,2,4]

Note. Mean values followed by different letters (a and b), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ([1, 2, 3, and 4]), in the same column, differ significantly ($P < .05$).

TABLE 7 Potassium (K) content of plant species, at various stages of growth (g kg^{-1} dry matter)

		Sampling dates							
Group	**Species**	**April 30**	**May 16**	**May 27**	**June 8**	**June 17**	**June 28**	**July 15**	**Mean**
Grasses	*A. gerardii*	–	14.1a[1,2]	13.9a[1]	11.6b[1]	11.0b[1]	11.9b[1]	7.7c[1,2]	11.9[1]
	S. pennata	–	9.5a[1]	10.4a[1]	11.9a,b[1]	13.6b[1]	11.8a,b[1]	4.7c[1]	11.2[1]
	P. alpinum	–	–	–	10.2a[1]	11.3a[1]	11.5a[1]	11.0a[1,2]	10.3[1]
Legumes	*T. repens*	–	13.1a[1,2]	17.1a[1]	14.8a[1]	14.2a[1]	14.1a[1]	9.3a[1,2]	14.1[1]
	L. aphaca	–	17.4a[1,2]	16.5a[1]	12.8a,b[1]	10.6a,b[1]	10.4a,b[1]	7.0b[1]	12.2[1]
	L. corniculatus	–	12.0a[1,2]	12.4a[1]	16.9a[1]	14.1a[1]	6.9a[1]	5.8a[1]	10.9[1]
Other forbs	*R. repens*	22.7a	13.8b[1,2]	14.1b[1]	11.5b[1]	17.0a,b[1]	13.8a,b[1]	–	15.1[1]
	A. millefolium	–	15.6a,b[1,2]	18.3a[1]	22.0a[2]	14.9a[1]	10.5b[1]	9.6b[1,2]	14.4[1]
	G. lucidum	–	20.1a[2]	16.4a,b[1]	14.3a,b[1]	12.6b[1]	15.8.a,b[1]	15.5a,b[2]	15.6[1]

Note. Mean values followed by different letters (a and b), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ([1, 2, 3, and 4]), in the same column, differ significantly ($P < .05$).

TABLE 8 Sodium (Na) content of plant species, at various stages of growth (g kg^{-1} dry matter)

		Sampling dates							
Group	**Species**	**April 30**	**May 16**	**May 27**	**June 8**	**June 17**	**June 28**	**July 15**	**Mean**
Grasses	*A. gerardii*	–	7.2a[1]	9.0a[1]	8.0a[1]	5.0b[1]	4.2b[1,3]	5.5b[1]	6.5[1]
	S. pennata	–	8.8a[1]	5.0b[1]	5.4b[1]	4.9b[1]	5.2b[1,3]	3.7b[1]	6.5[1]
	P. alpinum	–	–	–	5.0a[1]	8.9b[2]	7.9b[1,3]	6.5a,b[1]	7.5[1]
Legumes	*T. repens*	–	8.7a[1]	10.8a[1]	10.8a[1]	11.5a[2]	5.0a[1]	12.6a[1]	10.4[1,2]
	L. aphaca	–	6.5a[1]	9.8a[1]	4.5a[1]	17.3a[3]	15.7a[2,4,5]	16.6a[1]	11.8[1,2]
	L. corniculatus	–	7.8a[1]	7.9a[1]	7.8a[1]	3.5a[1]	17.2b[3,4,5]	5.2a[1]	8.0[1]
Other forbs	*R. repens*	36.5a	5.7b[1]	6.2b[1]	8.8b[1]	8.2b[2]	7.7b[1,2,4]	–	11.9[1,2]
	A. millefolium	–	19.0a,b[2]	11.3a[1]	16.7a,b[1]	10.5a,b[2]	22.8b[5]	10.7a[1]	15.3[2]
	G. lucidum	–	13.5a[1,2]	10.7a,b[1]	8.5b[1]	9.3a,b[2]	9.2a,b[1,4]	9.7a,b[1]	10.1[1,2]

Note. Mean values followed by different letters (a and b), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ([1, 2, 3, 4, and 5]), in the same column, differ significantly ($P < .05$).

TABLE 9 Iron (Fe) content of plant species, at various stages of growth (mg kg^{-1} dry matter)

		Sampling dates							
Group	**Species**	**April 30**	**May 16**	**May 27**	**June 8**	**June 17**	**June 28**	**July 15**	**Mean**
Grasses	*A. gerardii*	–	81.6a[1]	46.8a[1]	35.5a[1]	29.5a[1]	26.7a[1]	22.5a[1]	40.4[1,2]
	S. pennata	–	39.6a[1]	38.2a[1]	26.1a[1]	26.5a[1]	21.2a[1]	25.3a[1]	30.3[2]
	P. alpinum	–	–	–	17.7a[1]	34.4a[1]	30.4a[1]	35.4a[1]	30.7[2]
Legumes	*T. repens*	–	62.2a,b[1]	77.8a[1]	50.9b[1]	43.9b,c[1]	27.2c[1]	31.3b,c[1]	47.7[1,2]
	L. aphaca	–	70.3a[1]	55.9a[1]	34.5a[1]	35.5a[1]	27.9a[1]	33.1a[1]	41.3[1,2]
	L. corniculatus	–	89.6a[1]	38.4b[1]	43.55b[1]	28.2b[1]	33.6b[1]	35.3b[1]	44.8[1,2]
Other forbs	*R. repens*	120.1a	90.0a[1]	55.5a[1]	47.8a[1]	45.3a[1]	32.4a[1]	–	60.2[1]
	A. millefolium	–	72.4a[1]	50.2b[1]	43.1b,c[1]	23.9c,d[1]	20.7d[1]	23.4c,d[1]	36.4[1,2]
	G. lucidum	–	71.1a[1]	46.9a,b[1]	36.9b[1]	40.2b[1]	37.4b[1]	30.4b[1]	47.8[2]

Note. Mean values followed by different letters (a and b), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ([1] and [2]), in the same column, differ significantly ($P < .05$).

TABLE 10 Manganese (Mn) content of plant species, at various stages of growth (mg kg^{-1} dry matter)

Group	Species	Sampling dates							
		April 30	May 16	May 27	June 8	June 17	June 28	July 15	Mean
Grasses	*A. gerardii*	—	43.5a[1,2]	31.0a,b[1]	14.5b[1]	46.7a[1]	36.9a,b[1]	44.9a[1]	36.2[1,3]
	S. pennata	—	24.7a[1]	28.2a[1]	43.6a[2]	21.5a[2,3]	25.3a[1]	42.5a[1]	30.1[1,3,4]
	P. alpinum	—	—	—	21.5a[1,3]	35.9a[1,2]	42.8a[1]	45.6a[1]	37.9[1]
Legumes	*T. repens*	—	23.8[1]	16.4a[1]	18.5a[1]	18.8a[3]	14.6a[2]	23.6a[1]	18.7[2,3,4]
	L. aphaca	—	27.5a[1]	39.0a[1]	17.5a[1]	23.2a[1,2,3]	19.6a[1,2]	33.0a[1]	25.5[3,4,5]
	L. corniculatus	—	20.0a[1]	25.2a[1]	15.5a[1,4]	15.5a[3]	33.5a[1,2]	17.0a[1]	20.8[4,5]
Other forbs	*R. repens*	39.7a	32.4a[1]	26.0a[1]	35.0a[2,3]	19.3a[2,3]	18.5a[1]	—	29.2[1,5,6]
	A. millefolium	—	67.1a[2]	24.2b[1]	34.1a,b[2,3,4]	21.4b[2,3]	41.5a,b[1]	30.2a,b[1]	35.9[1,5]
	G. lucidum	—	14.5a[1]	17.7a[1]	23.5a[1,3]	27.8a[1,2,3]	18.5a[1,2]	19.0a[1]	20.5[4,6]

Note. Mean values followed by different letters (a and b), in the same row, differ significantly ($P < .05$). Mean values followed by different exponents ([1, 2, 3, 4, 5, and 6]), in the same column, differ significantly ($P < .05$).

TABLE 11 Copper (Cu) content of plant species, at various stages of growth (mg kg^{-1} dry matter)

Group	Species	Sampling dates							
		April 30	May 16	May 27	June 8	June 17	June 28	July 15	Mean
Grasses	*A. gerardii*	—	1.75[1]	2.2b[1]	1.5a[1]	1.5a[1]	1.5a[1]	0.7c[1]	1.5[1]
	S. pennata	—	5.6a[2]	1.5b[2]	1.0b[2]	1.5b[1]	2.2c[2]	1.0b[1]	2.6[1]
	P. alpinum	—	—	—	1.7a[1]	2.3b[2]	2.2b[2]	2.5b[2]	2.2[1]
Legumes	*T. repens*	—	2.5a,b	2.7a,b	2.4a	4.4b	2.7a,b	2.2a,b	2.9[1]
	L. aphaca	—	1.7a	2.3a	2.6a	7.6a	2.0a	5.1a	3.9[1,2]
	L. corniculatus	—	1.7a	2.0a	2.7a	2.7a	2.2a	1.8a	2.2[1]
Other forbs	*R. repens*	7.9a	3.2b	3.2b	2.5b	2.0b	2.0b	—	5.6[2]
	A. millefolium	—	6.0a	3.8a	2.7a	3.2a	8.7a	6.4a	3.4[1,2]
	G. lucidum	—	1.7a	1.7a	2.7a,b	4.0b	2.5a,b	2.2a,b	2.4[1]

Note. Mean values followed by different letters (a and b), in the same row, differ significantly ($P < .05$). Mean values followed by different exponent ([1] and [2]), in the same column, differ significantly ($P < .05$).

4 | DISCUSSION

The higher content of plant species of the botanical group of grasses in NDF and ADF, with statistically significant differences ($P < .05$) compared with the plant species of the botanical groups of legumes and other forbs observed in the present study, was due to the fact that the stems of the grasses contain a higher percentage of crude fiber than the other botanical groups, as well as a higher stem/leaf ratio (Pérez-Corona, Vázquez-de-Aldana, García-Criado, & García–Ciudad A., 1998; Vazquez-de-Aldana, García-Ciudad, Pérez-Corona, & García-Criado, 2000; Brueland, Harmoney, Moore, George, & Brummer, 2003; Kaya, Öncüer, & Ünal, 2004). Higher NDF and ADF content of grasses compared with legumes and other forbs, as well as higher NDF and ADF content of plant species at the growth stages in the ripening period, has also been reported by other investigators (Elgersma & Søegaard, 2016; Elgersma, Søegaard, & Jensen, 2013; Hejcman, Szaková, Schellberg, & Tlustoš, 2010; Nordheim-Viken, Volden, & Jørgensen, 2009).

The statistically significant differences ($P < .05$) observed in the NDF and ADF content of the plants during their different stages of growth, namely, the lower early stage content and the gradual increase as the plants mature, are due to the fact that as plants grow, leaf/stem ratio decreases, the rate of stems and shoots biomass increases, and, consequently, NDF and ADF content of plants is higher (Buxton, 1996; Ganskopp & Bohnert, 2001; González-Andrés & Ortiz, 1996). The lower NDF and ADF content of plants in the early stages of growth and its progressive increase as the plants grow has been reported by several investigators (Buxton & Redfearn, 1997; Tan et al., 2003).

The highest average content of the plant species of the botanical groups of legumes and other forbs in minerals, with statistically significant differences ($P < .05$) compared with the plant species of the botanical group of grasses, except for Mn, is due to the fact that the leaves have a higher mineral content than the stems (Myrvang et al., 2016; Tan et al., 1997) and that grasses have a higher stem/leaf ratio than other botanical groups (Buxton & Redfearn, 1997; Cherney, Mertens, & Moore, 1990). Generally, a higher mineral content of legumes and other forbs compared with grasses has also been reported by other researchers (Govasmark, Steen, Bakken, Strøm, & Hansen, 2005; Lindström, Frankow-Lindberg, Dahlin, Watson, &

Wivstad, 2014; Wyss & Kessler, 2002). Concerning the higher Mn content of the plant species of the botanical group of grasses compared with the plants of the other botanical groups, our results are in accordance with those of Hussain and Durrani (2008) and are in disagreement with those of Tufarelli et al. (2010a) and Pirhofer-Walzl et al. (2011).

The statistically significant differences ($P < .05$) observed in the mineral content of the plants studied during the various stages of growth are due to the fact that the leaves contain a higher percentage of minerals than the stems and that as the plants grow, leaf/stem ratio decreases (Arzani et al., 2004; Bovolenta, Spanghero, Dovier, Orlandi, & Clementel, 2008). Concerning the decrease in the mineral content observed in most of the plant species of the "Kostilata" grassland as the plants mature, the results of our study agree with those of Pirhofer-Walzl et al. (2011) and Schlegel et al. (2016). Different mineral contents between the different plant species, between the same plant species in different environments, and between the same plant species at different stages of growth have also been reported by other researchers (Elgersma & Søegaard, 2016; Lindström et al., 2014; Schlegel et al., 2016).

The mean NDF content of the plant species of the botanical group of grasses in the "Kostilata" grassland was lower than that of eight plant species of the botanical group of grasses in an experimental field in Italy, at an altitude of 1,500 m, whereas the average ADF content of the grasses in "Kostilata" grassland was in the intermediate range. The mean NDF and ADF content of the plant species of the botanical group of other forbs of "Kostilata" grassland was higher than that of three plant species of the botanical group of other forbs in the above experimental field. The mean NDF content of *P. alpinum* L. in the "Kostilata" grassland was lower than that of *P. alpinum* L. in the experimental field of Italy, whereas the ADF contents of the two plant species were similar. The mean NDF and ADF content of *T. repens* L. in "Kostalata" grassland was higher than that of *T. repens* L. in the Italian experimental field (Bovolenta et al., 2008).

The mean NDF and ADF content of *S. pennata* L. in the "Kostalata" grassland was lower than that reported by Hussain and Durrani (2009). The mean NDF content of *T. repens* L. in the "Kostilata" grassland compared with the NDF content of *T. repens* L. at the flowering stage in a grassland in Turkey was higher, whereas its ADF content was lower (Kiraz, 2011). The mean NDF content of *L. aphaca* L. in the "Kostilata" grassland was higher than that of *L. aphaca* L. in grasslands of Southeastern Anatolia Region of Turkey (Basbag, Aydin, Çaçan, & Sayar, 2012). At a grassland in Italy at an altitude of 1,300 to 1,500 m, the values of NDF and ADF content of four legumes were slightly lower than those of the "Kostilata" grassland legumes. The values of NDF and ADF contents of the other forbs of "Kostilata" grassland were intermediate compared with the values of five other forbs in the Italian grassland. In the same grassland, *L. corniculatus* L. and *A. millefolium* L. showed lower NDF and ADF values than those of the corresponding plant species of "Kostilata" grassland (Tufarelli, Cazzato, Ficco, & Laudadio, 2010b). The NDF and ADF content of the other forbs of "Kostilata" grassland compared with the content of five other forbs in a grassland in Rieti, central Italy, at altitudes between 400 and 500 m, showed intermediate values (Tufarelli et al., 2010a). In May, the NDF and ADF contents of *T. repens* L. and *L. corniculatus* L. of "Kostilata" grassland were higher than those of the corresponding plant species at Research Farm Foulumgaard in Denmark (Elgersma & Søegaard, 2016), and these differences can probably be attributed to different climatic and soil conditions.

The Ca and K contents of the grasses of "Kostilata" grassland were higher than those of lower altitude grasslands in northwestern Greece, near the area of our research, whereas the P and Mg contents were similar. The legumes of "Kostilata" grassland had a higher Ca and K content, whereas the P and Mg contents were lower. The other forbs of "Kostilata" grassland had higher Ca content, whereas the P, Mg, and K contents were similar (Roukos et al., 2011).

The content of all nine plant species of "Kostilata" grassland in Ca, Fe, and Mn was lower, compared with the Ca, Fe, and Mn content of three grasses, four legumes, and eight other forbs found in two grasslands in central Italy, whereas their content in P was higher. The grasses of all the grasslands had similar Mg content, whereas the legumes of "Kostilata" had a higher content, and the other forbs of "Kostilata" had intermediate content, compared with those of the corresponding plants in the Italian grasslands. Concerning K, the grasses of all the grasslands had similar content, whereas the legumes and the other forbs of "Kostilata" grassland had intermediate content. Regarding Na, the grasses of "Kostilata" grassland had slightly higher content and the legumes and other forbs also had a higher content (Tufarelli et al., 2010a, 2010b).

Concerning the average content of grasses in Mg, Ca, and K in relation to the average content of legumes and other forbs, our results are in agreement with those reported by Elliot (1986) and McDowell (2003), while disagreeing with the results for P and Na. Our results regarding the content of other forbs in Ca, P, and K, compared with the corresponding content of legumes, are in agreement with the results of the above researchers, while disagreeing with their results regarding the Mg content.

The results of the present study on the Mn mean content of plant species are in agreement with those reported by Pirhofer-Walzl et al. (2011) in the fact that grasses have a higher Mn content compared with legumes and other forbs and disagree on the Mn content of legumes compared with that of other forbs. Moreover, higher levels of Mn in grasses compared with other plant species were also reported by Hussain and Durrani (2008).

Concerning variations in the content of plant species in Ca, the results of our study agree with those of Tan et al. (2003) with respect to *A. gerardii* V., *S. pennata* L., and *G. lucidum* L. As regards the P content, our results are consistent with those of Tan et al. for all plant species, except *S. pennata* L. and *L. corniculatus* L. As far as Mg content is concerned, our results are consistent with the results of Tan et al. only for *S. pennata* L. and of *A. millefolium* L. Finally, about variations in K content, our results are consistent with those of the above researchers for all plant species, except *P. alpinum* L.

Our results on the variation of Ca content disagree with those reported by Mountousis et al. (2008)b) for *L. aphaca* L., *R. repens* L.,

and *A. millefolium* L. and are in agreement with the results for the other plant species. As for the P content, our results disagree with the results of Mountousis et al. for all plant species. In addition, with regard to variation in Mg content, our results disagree with the results for *A. gerardii* V., *P. alpinum* L., and *L. corniculatus* L. and are in agreement with those for the other plant species. Concerning the Na content, our results disagree with those for *P. alpinum* L., *T. repens* L., and *L. aphaca* L. and agree with the above researchers for the other plant species.

Compared with a study in an Italian grassland, the mean Ca, Fe, Mn, and Cu contents of *L. corniculatus* L. and *A. millefolium* L. were lower in "Kostilata" grassland, whereas the P and Na content in both plant species was higher in the grassland of "Kostilata." The Mg content in *L. corniculatus* L. was higher in the grassland of "Kostilata," whereas in *A. millefolium* L. was lower. The K content in *L. corniculatus* L. was similar in both grasslands, whereas *A. millefolium* L. had a lower K content in the grassland of "Kostilata" (Tufarelli et al., 2010b).

A. millefolium L. showed a higher Ca and K content and slightly lower Mg, Mn, Cu, and Fe contents in the grassland of "Kostilata," compared with the corresponding contents of *A. millefolium* L. before its flowering stage in Bragança, Portugal (Dias et al., 2016).

The content of *T. repens* L. and *L. corniculatus* L. in P, Mg, K, Fe, Mn, and Cu in the "Kostilata" grassland was lower, whereas in Na was higher than the corresponding content of the same plant species in Research Farm Foulumgaard, Aarhus University, Denmark, differences that can obviously be attributed to different climatic and soil conditions (Pirhofer-Walzl et al., 2011).

The average Ca, P, and Mg contents of *L. aphaca* L. in the "Kostilata" grassland were lower than those in a grassland in Turkey (Basbag et al., 2012).

The differences observed in both the content and the variation in the chemical composition between the plant species of the "Kostilata" grassland, compared with other plant species from other grasslands, can be mainly attributed to the different climatic conditions prevailing between the ecosystems (Flisch et al., 2009; Georgievskki et al., 1982; Öborn et al., 2008; Ramírez-Pérez et al., 2000).

The nine plant species we have studied, namely, the three most predominant of each botanical group, constitute the bulk of the grazing material of the grassland under investigation. Their nutritional value is good, as their NDF content does not exceed 600 g kg^{-1} DM during the study period. When the NDF content of forage exceeds 600 g kg^{-1} DM, there is a decrease in food consumption by animals (Mertens, 1994). Also, the mineral content of almost all plants meets the nutritional requirements of sheep, according to National Research Council (1985). Specifically, all plant species meet the nutritional requirements of the animals for K and Na. All plant species meet the needs of animals for Ca, except for *P. alpinum* L. Only other forbs and legumes meet the maintenance requirements in Mg, whereas only grasses and other forbs meet the requirements in P. As far as micronutrients are concerned, all plants meet the animal requirements for Fe and Mn, whereas no plants meet the requirements for Cu.

Among the plant species, those belonging to the botanical groups of legumes and other forbs have the best nutritional value. Therefore, proper grazing management should be applied to "Kostilata" grassland, so that the above botanical groups are preserved in the vegetation composition. According to Papanastasis et al. (2002), overgrazing of sheep in subalpine grasslands reduces plant diversity, especially in other forbs and legumes.

5 | CONCLUSION

The same plant species have different chemical compositions in different environments. The predominant plant species of the "Kostilata" grassland, during the research period, generally had good nutritional value. Their content in NDF and ADF was generally satisfactory. Most of the plant species cover the needs of sheep for trace element and macroelement. Among the plant species, legumes and other forbs had the highest nutritional value.

The variation of the chemical composition of the dominant plant species of the subalpine grassland of "Kostilata" provides forage of good nutritional value, contributing to the preservation of nomadic farming in the area.

ACKNOWLEDGEMENTS

This study was supported and cofinanced by the European Union (European Fund of Regional Development) and National resources in the framework of the Regional Operational Program "Thessaly - Central Greece – Epirus."

DATA AVAILABILITY STATEMENT

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

ORCID

Charalampos Koutsoukis https://orcid.org/0000-0003-3473-2958
Konstantoula Akrida-Demertzi https://orcid.org/0000-0002-9904-7700

REFERENCES

AOAC. (1997). *Official methods of analysis (method 973.18)*, 16th Ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA (3rd rev.).

AOAC. (1999). *Official methods of analysis (method 968.08)*, 16th Ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA (5th rev.).

Arzani, H., Zohdi, M., Fish, E., Zahedi Amiri, G., Nikkhah, A., & Wester, D. (2004). Phenological effects on forage quality of five grass species. *Journal of Range Management*, *57*, 624–629. https://doi.org/10.2307/4004019

Basbag, M., Aydin, A., Çaçan, E., & Sayar, M. S. (2012). Determination of quality characters of some grass pea taxa (*Lathyrus* spp.) collected in natural areas of Southeastern Anatolia Region of Turkey. *Tarım Bilimleri Araştırma Dergisi (Abstract in English)*, *5*(2), 111–114.

Belkhir, S., Koubaab, A., Khadhria, A., Ksontinic, M., Nadjid, H., Smitia, S., & Stevanovicd, T. (2013). Seasonal effect on the chemical composition of the leaves of *Stipa tenacissima* L. and implications for pulp properties. *Industrial Crops and Products*, *44*, 56–61. https://doi.org/10.1016/j.indcrop.2012.09.023

Bovolenta, S., Spanghero, M., Dovier, S., Orlandi, D., & Clementel, F. (2008). Chemical composition and net energy content of alpine pasture species during the grazing season. *Animal Feed Science and Technology*, *146*, 178–191. https://doi.org/10.1016/j.anifeedsci.2008.06.003

Brueland, B. A., Harmoney, K. R., Moore, K. J., George, J. R., & Brummer, E. C. (2003). Development morphology of smooth bromegrass growth following spring grazing. *Crop Science*, *43*, 1789–1796.

Buchin, S., Martin, B., Dupont, D., Bornard, A., & Achilleos, C. S. (1999). Influence of the composition of Alpine highland pasture on the chemical, rheological and sensory properties of cheese. *Journal of Dairy Research*, *66*, 579–588. https://doi.org/10.1017/s0022029999003842

Buxton, D. R. (1996). Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology*, *59*, 37–49.

Buxton, D. R., & Redfearn, D. D. (1997). Plant limitations to fiber digestion and utilization. *Journal of Nutrition*, *127*, 814–818.

Chatzitheodoridis, F., Michailidis, A., & Theodossiou, G. (2007). Comparative analysis of sheep–goat farming in a typical Greek island: *Economy and environment*. *Applied Economics and Policy Analysis*, *1*(1-2), 191–200.

Cherney, D. J. R., Mertens, D. R., & Moore, J. E. (1990). Intake and digestibility by withers as influenced by forage morphology at three levels of forage offering. *Journal of Animal Science*, *68*(12), 4387–4399. https://doi.org/10.2527/1990.68124387x

Corah, L. R., & Dargatz, D. (1996). *Forage analyses from cow/calf herds in 18 states*. In: Beef Cow/Calf Health and Productivity Audit (CHAPA). Veterinary Services, National Health Monitoring System, USDA, Washington, DC: USDA Animal and Plant Health Inspection Service.

Cosentino, S. L., Porqueddu, C., Copani, V., Patanè, C., Testa, G., Scordia, D., & Melis, R. (2014). European grasslands overview: Mediterranean region grassland. *Science in Europe*, *19*, 41–56.

Deinum, B., & Maassen, A. (1994). Effects of drying temperature on chemical composition and in vitro digestibility of forages. *Animal Feed Science and Technology*, *46*, 75–86.

Dias, M. I., Morales, P., Barreira, J. C. M., Oliveira, M. B. P. P., Cortes Sánchez-Mata, M., & Ferreira, I. C. F. R. (2016). Minerals and vitamin B9 in dried plants vs. infusions: Assessing absorption dynamics of minerals by membrane dialysis tandem in vitro digestion. *Food Bioscience*, *13*, 9–14.

Dimopoulos, P., Raus, T., Bergmeier, E., Constantinidis, T., Iatrou, G., Kokkini, S., … Tzanoudakis, D. (2013). *Vascular plants of Greece. An annotated checklist*. Berlin & Athens: Botanic Garden and Botanical Museum Berlin-Dahlem & Hellenic Botanical Society.

Earle, K. E., Kienzle, E., Opitz, B., Smith, P. M., & Maskell, I. E. (1998). Fibre affects digestibility of organic matter and energy in pet foods. *The Journal of Nutrition*, *128*(12), 2798–2800.

Elgersma, A., & Søegaard, K. (2016). Changes in nutritive value and herbage yield during extended growth intervals in grass–legume mixtures: Effects of species, maturity at harvest, and relationships between productivity. *Grass and Forage Science*, *2016*, 78–93.

Elgersma, A., Søegaard, K., & Jensen, S. K. (2013). Herbage dry-matter production and forage quality of three legumes and four non-leguminous forbs grown in single-species stands. *Grass and Forage Science*, *69*, 705–716.

Elliot, R. (1986). The use of green forage material to improve the nutritional status of animals fed low quality crop residues. In R. M. Dixon (Ed.), *Ruminant feeding systems utilizing fibrous agricultural residues*. Australia: Camberra, ACT.

Flisch, R., Sinaj, S., Charles, R., & Richner, W. (2009). Grundlagen für die Düngung im Acker-und Futterbau. *Agrarforschung*, *16*, 1–100.

Foroughbakhch, R., Hernandez-Pinero, J. L., & Carrillo-Parra, A. (2012). Nutrient profile, floristic compositions and preference index of shrubs and herbs consumed by goats in semiarid region of Northeastern Mexico. *Journal of Animal and Veterinary Advances*, *11*, 1299–1305.

Ganskopp, D., & Bohnert, D. (2001). Nutritional dynamics of 7 northern Great Basin grasses. *Journal of Range Management*, *54*, 640–647.

Georgievskki, V. I., Annenkov, B. N., & Samokhin, V. Y. (1982). *Mineral nutrition of animals*. London: Butterworths.

González-Andrés, F., & Ortiz, J. M. (1996). Potential of Cytisus and allied genera (Genisteae: Fabaceae) as forage shrubs. 2. Chemical composition of the forage and conclusions. *New Zealand Journal of Agricultural Research*, *39*, 205–213.

Govasmark, E., Steen, A., Bakken, A. K., Strøm, T., & Hansen, S. (2005). Factors affecting the concentration of Zn, Fe and Mn in herbage from organic farms and in relation to dietary requirements of ruminants. *Acta Agriculturae Scandinavica*, *55*(2), 131–142.

Greene, L. W. (2000). Designing mineral supplementation of forage programs for beef cattle. *Journal of Animal Science*, 77 (E-Suppl.), 1-9.

Greene, L. W., Baker, J. F., & Hardt, P. F. (1989). Use of animal breeds and breeding to overcome the incidence of grass tetany: A review. *Journal of Animal Science*, *67*, 3463–3469.

Hale, C., & Olson, K. C. (2001). *Mineral supplements for beef cattle*. Columbia: Missouri Extension Service. MU. Publication G2081.

Hejcman, M., Szaková, J., Schellberg, J., & Tlustoš, P. (2010). The Rengen Grassland Experiment: Relationship between soil and biomass chemical properties, amount of elements applied, and their uptake. *Plant and Soil*, *333*, 163–179.

Hussain, F., & Durrani, M. J. (2008). Mineral composition of some range grasses and shrubs from Harboi rangeland Kalat, Pakistan. *Pakistan Journal of Botany*, *40*(6), 2513–2523.

Hussain, F., & Durrani, M. J. (2009). Nutritional evaluation of some forage plants from Harboi rangeland, Kalat, Pakistan. *Pakistan Journal of Botany*, *41*(3), 1137–1154.

Kaya I., Öncüer A. & Ünal Y. (2004). Nutritive value of pastures in Kars district. I. Botanical and nutrient composition at different stages of maturity. *Turkish Journal of Veterinary and Animal Sciences*, *28*, 275-280.

Kiraz, A. B. (2011). Determination of relative feed value of some legume hays harvested at flowering stage. *Asian Journal of Animal and Veterinary Advances*, *6*(5), 525–530.

Leng, R. A. (1990). Factors affecting the utilization of 'poor-quality' forage by ruminants particularly under tropical conditions. *Nutritional Research Review*, *3*, 277–303.

Lindström, B. E. M., Frankow-Lindberg, B. E., Dahlin, A. S., Watson, C. A., & Wivstad, M. (2014). Red clover increases micronutrient concentrations in forage mixtures. *Field Crops Research*, *169*, 99–106.

McCollum, F. T., Galyean, M. L., Krysl, L. J., & Wallace, J. D. (1985). Cattle grazing blue grama rangeland I. *Seasonal diets and rumen fermentation*. *Journal of Range Management*, *38*, 539–543.

McDowell, L. R. (2003). *Minerals in animal and human nutrition* (2nd ed.). Amsterdam: Elsevier.

McDowell, L. R., Ellis, G. L., & Conrad, J. H. (1984). Mineral supplementation for grazing cattle in tropical regions. *World Animal Review*, *52*, 1–12.

McDowell, L. R., & Valle, G. (2000). In D. I. Givens, E. Owen, R. F. E. Axford, & H. M. Omed (Eds.), *Major minerals in forages*. New York: Forage Evaluation in Ruminant Nutrition, CABI Publishing.

McMorran, R., Santini, F., Guri, F., Gomez-y-Paloma, S., Price, M., Beucherie, O., … Cloye, G. (2015). A mountain food label for Europe? The role of food labelling and certification in delivering sustainable development in European mountain regions. *Journal of Alpine Research*, *103*, 1–22.

Mertens, D. R. (1994). Regulation of forage intake. In G. C. Fahey, M. Collins, D. R. Mertens, & L. E. Moser (Eds.), *Forage quality, evaluation, and utilization*. ASA, CSSA and SSSA. (pp. 450–493). Wisconsin: Madison.

Moreira, F. B., Prado, I. N., Cecato, U., Wada, F. Y., & Mizubuti, I. Y. (2004). Forage evaluation, chemical composition, and in vitro digestibility of continuously grazed star grass. *Animal Feed Science and Technology*, *113*, 239–249.

Mountousis, I., Papanikolaou, K., Stanogias, G., Chatzitheodoridis, F., & Roukos, C. (2008). Seasonal variation of chemical composition and dry matter digestibility of rangelands in NW Greece. *Journal of Central European Agriculture*, *9*(3), 547–556.

Mountousis, I., Papanikolaou, K., Stanogias, G., Roukos, C., Chatzitheodoridis, F., & Papazafiriou, A. (2008). Monthly variations of mineral content in semi-arid rangelands in western Macedonia, Greece. *Bulgarian Journal of Agricultural Science*, *14*(4), 361–372.

Myrvang, M. B., Hillersøy, M. H., Heim, M., Bleken, M. A., & Gjengedal, E. (2016). Uptake of macro nutrients, barium, and strontium by vegetation from mineral soils on carbonatite and pyroxenite bedrock at the Lille bukt Alkaline Complex on Stjernøy, Northern Norway. *Journal of Plant Nutrition and Soil Science*, *179*, 705–716.

National Research Council (1985). *Nutrients requirements of sheep* (6th revised ed.). Washington, DC, USA: National Academy Press.

Nordheim-Viken, H., Volden, H., & Jørgensen, M. (2009). Effects of maturity stage, temperature and photoperiod on growth and nutritive value of timothy (*Phleum pratense* L.). *Animal Feed Science and Technology*, *152*, 204–218.

Noziere, P., Graulet, B., Lucas, A., Martin, B., Grolier, P., & Doreau, M. (2006). Carotenoids for ruminants: From forages to dairy products. *Animal Feed Science and Technology*, *131*, 418–450.

Öborn, I., Bengtsson, H., Gustafson, G. M., Holmqvist, J., Modin-Edman, A. K., Nilsson, S. I., … Jonsson, S. (2008). Farm-scale nutrient and trace element dynamics in dairy farming. In P. Schlegel, S. Durosoy, & A. W. Jongbloed (Eds.), *Trace elements in animal production systems*. The Netherlands: Wageningen Academic Publishers, Wageningen.

Papanastasis, V., & Ispikoudis, I. (2013). *Rangeland ecology*. Thessaloniki (in Greek): Giachoudis publications.

Papanastasis, V., & Karagiannakidou - Papadimitriou K. (1993). *The most important grasses of natural grasslands*. Athens: Ministry of Agriculture. (in Greek)

Papanastasis V. P., Vrachnakis M., Iovi K., Platis P., Karalazos A. & Hatziminaoglou I. (2002). Dynamics of herbage production in a grazed of mountain grassland in Greece. Proceeding of the 19th General Meeting of the European Grassland Federation. Multifunction Grasslands: Quality Forage, Animal Products and Landscapes, La Rochelle France.

Peeters, A. (2008). Challenges for grassland-based systems and their production potential in Europe *Grassland*. *Science in Europe*, *13*, 9–24.

Pérez-Corona, M. E., Vázquez-de-Aldana, B. R., García-Criado, B., & García–Ciudad A. (1998). Variations in nutritional quality and biomass production of semiarid grasslands. *Journal of Range Management*, *51*, 570–576.

Peyraud, J., & Peeters, L. A. (2016). The role of grassland based production system in the protein security. *Grassland Science in Europe*, *21*, 29–44.

Pirhofer-Walzl, K., Søegaard, K., Høgh-Jensen, H., Eriksen, J., Sanderson, M. A., & Rasmussen, J. (2011). Forage herbs improve mineral composition of grassland herbage. *Grass and Forage Science*, *66*, 415–423.

Provenza, F. D. (1995). Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management*, *48*, 2–17.

Ramírez-Pérez, A. H., Buntinx, S. E., & Rosiles, R. (2000). Effect of breed and age on the voluntary intake and the micro mineral status of non-pregnant sheep. *Small Ruminant Research*, *37*, 231–242. https://doi.org/10.1016/s0921-4488(00)00121-8

Rook, A. J., Dumont, B., Isselstein, J., Osoro, K., Wallis De Vries, M. F., Parente, G., & Mills, J. (2004). Matching type of livestock to desired biodiversity outcomes in pastures. *Biological Conservation*, *119*(2), 137–150. https://doi.org/10.1016/j.biocon.2003.11.010

Roukos, C., Koutsoukis, C., Akrida-Demertzi, K., Karatassiou, M., Demertzis, G. P., & Kandrelis, S. (2016). The effect of altitudinal zone on soil properties, species composition and forage production in a subalpine grassland in northwest Greece. *Applied Ecology and Environmental Research*, *15*(1), 609–626.

Roukos, C., Papanikolaou, K., Karalazos, A., Chatzipanagiotou, A., Mountousis, I., & Mygdalia, A. (2011). Changes in nutritional quality of herbage botanical components on a mountain side grassland in North-West Greece. *Animal Feed Science and Technology*, *169*(1-2), 24–34.

Schlegel, P., Wyss, U., Arrigo, Y., & Hess, H. D. (2016). Mineral concentrations of fresh herbage from mixed grassland as influenced by botanical composition, harvest time and growth stage. *Animal Feed Science and Technology*, *219*, 226–233.

Strid, A. (1986). *Mountain flora of Greece* (Vol. 1). Press, Cambridge: Cambridge Univ.

Strid, A., & Tan, K. (1991). *Mountain flora of Greece* (Vol. 2). Press, Edinburgh: Edinburgh Univ.

Tajeda, R., Mcdowell, L. R., Martin, F. G., & Conrad, J. H. (1985). Mineral element analyses of various tropical forages in Guatemala and their relationship to soil concentrations. *Nutrition Reports International*, *32*, 313–324.

Tamburino, R., Guida, V., Pacifico, S., Rocco, M., Zarelli, A., Parente, A., & Di Maro, A. (2012). Nutritional values and radical scavenging capacities of grass pea (*Lathyrus sativus* L.) seeds in Valle Agricola district Italy. *Australian Journal of Crop Science*, *6*(1), 149–156.

Tan, M., Bakoglu, A. & Koc, A. (1997). The changing of aboveground biomass and chemical composition of birds food trefoil (*Lotus corniculatus* L.) during growing period. 2nd Field Crops Congress in Turkey, Samsun.

Tan, M., Temel, S. & Yolcu, H. (2003). Effects of harvest management on the mineral composition of common vetch. Proceedings of the 12th Symposium of the European Grassland Federation, Pleven, Bulgaria.

Tufarelli, V., Cazzato, E., Ficco, A., & Laudadio, V. (2010a). Assessing nutritional value and in vitro digestibility of Mediterranean pasture species using yak (Bos grunniens) faeces as alternative microbial inoculum in a Daisy incubator. *Journal of Food, Agriculture & Environment*, *8*(2), 477–481.

Tufarelli, V., Cazzato, E., Ficco, A., & Laudadio, V. (2010b). Evaluation of Chemical composition and *in vitro* digestibility of Appennine pasture plants using yak (*Bos grunniens*). Rumen fluid or faecal extract as inoculum source. *Asian-Australasian Journal of Animal Sciences*, *23*(12), 1587–1593.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3584–3597.

Vazquez-de-Aldana, B. R., García-Ciudad, A., Pérez-Corona, M. E., & García-Criado, B. (2000). Nutritional quality of semi-arid grassland in western Spain over a 10-year period: Changes in chemical composition of grasses, legumes and forbs. *Grass and Forage Science*, *55*, 209–220.

Viallon, C., Martin, B., Verdier-Metz, I., Pradel, P., Garel, J.-P., Coulon, J. B., & Berdague, J. L. (2000). Transfer of monoterpenes and sesquiterpenes from forages into milk fat. *Lait*, *80*, 635–641.

Vogel, K. P., Pedersen, J. F., Masterson, S. D., & Toy, J. J. (1999). Evaluation of a filter bag system for NDF, ADF and IVDMD forage analysis. *Crop Science*, *39*, 276–279.

Vrachnakis, M. (2015). Rangeland, Digital textbook. https://repository.kallipos.gr/bitstream/11419/1191/2/00_master_document_%CE%9A%CE%9F%CE%A5.pdf (in Greek).

Ward, M., & Lardy, G. (2005). *Beef cattle mineral nutrition*. North Dakota State University: Publ. AS-1287. NDSU Extension Service.

Wyss, U., & Kessler, J. (2002). The intensity of grassland management influences the mineral contents of the grass. *Agrarforschung*, *9*, 292–297.

Zervas, G. (1998). Quantifying and optimizing grazing regimes in Greek mountain systems. *Journal of Applied Ecology*, *35*, 983–986.

Zervas, G. (2013). *Feeding of ruminants*. Thessaloniki (in Greek): Stamoulis publications.

How to cite this article: Koutsoukis C, Roukos C, Demertzis PG, Kandrelis S, Akrida-Demertzi K. The variation of the chemical composition of the main plant species in a subalpine grassland in northwestern Greece. *Legume Science*. 2019;1:e23. https://doi.org/10.1002/leg3.23