

Genetic diversity of *Thymus sibthorpii* Bentham in mountainous natural grasslands of Northern Greece as related to local factors and plant community structure

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ABSTRACT

The local scale genetic diversity of *Thymus sibthorpii*, an aromatic species of the Greek flora, was studied both in relation to its habitat characteristics. The study was conducted in Cholomontas mountain, northern Greece at altitudes of 760–870 m. Genetic material from Southern and Northwestern exposures and from different grazing intensities within each exposure, was studied using ISSR molecular markers. The plant cover at each study site was measured and the floristic composition of the site was calculated. The grazing intensity affected the species composition and reduced the abundance of *T. sibthorpii*. The floristic diversity in terms of species richness, evenness and Shannon index (I_p) was higher at the southern sites. Within population gene diversity (H_p) ranged from 0.203 to 0.245 and Shannon index (I_c) from 0.311 to 0.377. Most of the genetic diversity resided within populations (88%). Significant genetic differentiation was detected among the populations from the southern and northwestern sites, as well as among populations of each exposure. Genetic diversity of *T. sibthorpii* was not correlated to species diversity (richness and evenness), either within or between populations. Instead, genetic diversity was found to be positively correlated with environmental factors, such as N soil content and soil moisture.

1. Introduction

Grazing intensity (Dumont et al., 2009) and environmental site conditions (De Bello et al., 2005), are the main ecosystem determinants that shape plant community structure in terms of species composition and diversity. The effects of grazing intensity on plant community structure vary among the grasslands depending on how grazing intensity interacts with characteristics of the ecosystems such as primary productivity, vegetation composition and evolutionary history (Huston, 1979; Milchunas et al., 1988; Milchunas and Lauenroth, 1993; Noy-Meir, 1995; Proulx and Mazumder, 1998; Lezama et al., 2014).

Plant community structure in grasslands is also controlled by environmental conditions such as topography, climate and soil. Topography (elevation, slope and exposure) shape the micro-climate at a local scale. In this regard, Wright et al. (2003) have noted that plant

community structure in grasslands is mainly influenced by local factors, and only secondarily by factors operating at the landscape level. Among the topography parameters, the spatial distribution of vegetation is mainly affected by slope exposure (Kutiel, 1992). Generally, the south-facing slopes tend to be drier and warmer compared to north ones as they receive higher solar radiation resulting in higher evapotranspiration rates and higher daily maximum temperatures, mainly during summer (Kutiel and Lavee, 1999). This differentiation affects plant communities, especially in Mediterranean areas, where water availability is a determinant factor for plant growth (Badano et al., 2005).

Similar to plant communities structure, the genetic diversity of grassland plant species could be affected both by management practices, such as grazing, and environmental factors as well (Last et al., 2014; Abraham et al., 2015). The balance between genetic drift, inbreeding, recombination, gene flow, mutation, and selection (Loveless

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Table 1

General location, habitat characteristics, grazing intensity and soil features recorded in each area and used for the multivariate analyses.

Sites	Altitude (m)	Longitude	Latitude	Area (ha)	No*	Dominant herbaceous species	Explanatory variables						
							Habitat	FUP	Soil Features				
									pH	Or.M (%)	Soil moisture	N (%)	P (mg/100gr)
S1	797	23°32'44"	40°26'86"	1.5	10	<i>Trifolium cherleri</i> , <i>Thymus sibthorpii</i> , <i>Cynodon dactylon</i>	S	0.50	4.4	7.9	7.7	0.10	2.0
S2	804	23°30'11"	40°25'94"	1.0	10	<i>Thymus sibthorpii</i> , <i>Vulpia sp.</i> , <i>Cynosurus echinatus</i>	S	0.40	4.8	7.4	5.1	0.12	1.6
S3	772	23°30'00"	40°24'28"	2.0	20	<i>Plantago lanceolata</i> , <i>Vulpia sp.</i> , <i>Trifolium repens</i>	S	0.70	4.5	10.0	8.9	0.20	1.1
NW1	799	23°29'83"	40°24'61"	1.0	10	<i>Agrostis canina</i> , <i>Thymus sibthorpii</i> , <i>Cynodon dactylon</i>	NW	0.55	4.5	10.3	8.5	0.11	1.1
NW2	765			1.0	10	<i>Agrostis sp.</i> , <i>Pteridium sp.</i> , <i>Plantago lanceolata</i> ,	NW	0.40	4.8	9.3	6.5	0.07	1.3
NW3	780	23°29'67"	40°25'96"	2.1	20	<i>Agrostis sp.</i> , <i>Rubus sp.</i> , <i>Thymus sibthorpii</i>	NW	0.00	4.4	7.1	8.5	0.07	1.3
NW4	795	23°29'91"	40°25'98"	1.5	10	<i>Cynodon dactylon</i> , <i>Lotus corniculatus</i> , <i>Plantago lanceolata</i>	NW	0.65	4.7	10.8	15.0	0.08	1.3
NW5	805	23°29'06"	40°26'07"	1.0	10	<i>Cynodon dactylon</i> , <i>Holcus mollis</i> , <i>Thymus sibthorpii</i>	NW	0.45	4.5	9.0	11.2	0.09	2.1

S: South slopes, NW: Northwest slopes, FUP: Forage Utilization Percentage according to the formula [(Dry matter production of ungrazed plots – Dry matter production of grazed plots)/ Dry matter production of ungrazed plots]*100, Org.M (%): Organic Matter (%) *Number of individual plants of *Thymus sibthorpii* that were tested by ISSRs.

and Hamrick, 1984) that control genetic diversity at the intra and inter population levels depends on the mode of reproduction or the life form of the plant species (Hamrick and Godt, 1996), while also on habitat biotic and abiotic characteristics. The effect of grazing could be negative, as grazing could reduce plant population size (Aguado-Santacruz et al., 2004; Fu et al., 2005), or positive as seed dispersal among populations could be increased by grazing animals (Jacquemyn et al., 2010). There is evidence that the type of the effect is probably related to grazing intensity (Ma et al., 2014).

Theoretically, species diversity and genetic diversity could be correlated due to their similar response to the local factors and/or to their interaction (Vellend and Geber, 2005). However, contradictory results have been reported about this correlation for various plant species and habitats at a local or large scale. Particularly, there are reports for positive (He et al., 2008; He and Lamont, 2010; Odat et al., 2010), negative (Silvertown et al., 2009; Nestmann et al., 2011), and no correlation (Odat et al., 2004; Abraham et al., 2015) at a local scale. These contradictory results reflect the complex indirect relationships between genetic and species diversity, which are mediated by environmental differences of the habitat (Odat et al., 2010).

The aim of the present study was to evaluate the abundance and genetic diversity of the sub-shrub *Thymus sibthorpii*, in relation to plant community structure, environmental factors (slope, exposure and soil), and management (grazing), at a local level, in mountainous grasslands of Northern Greece. *Thymus sibthorpii* is an aromatic cross-pollinated diploid (2n = 28) (Strid et al., 1981; Markova and Goranova, 1994), perennial sub-shrub, part of the section Serpyllum (one of the eight sections of the genus *Thymus*), native to Balkan Peninsula (Sáez and Stahl-Biskup, 2002). Particularly, its population occurrence was reported in the floristic regions of North East (NE), North central and East central Greece along with South Pindos and the North Aegean islands of Thasos and Samothrace (Koureas, 2012). Generally, *Thymus* species are used for medicinal and culinary purposes worldwide (Sáez and Stahl-Biskup, 2002), mainly due to their bioactive compounds (essential oil, polyphenols etc.) that present excellent biological activities (Nickavar et al., 2005). The essential oil of thyme has strong antimicrobial, insecticidal, antifungal, antiviral and antioxidant properties related to the rich content of phenolic compounds such as thymol and/or carvacrol (Dababneh, 2007; Jamali et al., 2012). As a consequence, it is broadly used in food, cosmetic and pharmaceutical industries. Thyme is a widespread genus (Sáez and Stahl-Biskup, 2002) characterized by good

adaptability to different soil and climatic conditions.

The majority of the research regarding the effect of grazing on the genetic diversity of grassland plant species focus on palatable species. *T. sibthorpii* is a significant aromatic and medicinal species, but also a less-preferable by the grazing animals. However, as the natural populations are the main source of its supply, they are usually threatened by the human activities, such as overgrazing and overharvesting. *Thymus albicans* is considered an endangered species in Spain (Girón et al., 2012) as well as several species of *Thymus* in the Mediterranean region of Turkey (Karaca et al., 2015). In this respect, it is essential to study population genetic diversity and the factors that shape it, in order to achieve sustainable management and conservation. The following questions were addressed: 1) Is there any differentiation in species composition and *T. sibthorpii* abundance between south and north slope exposures? 2) Is there any genetic differentiation between the populations of *T. sibthorpii* from south and northwest slope exposures? 3) Is there any effect of grazing on the species composition and the genetic diversity of *T. sibthorpii*? 4) Is the genetic diversity of *T. sibthorpii* correlated to species composition and species diversity among and within habitats?

2. Materials and methods

2.1. Study sites and plant material

The research was carried out in the Taxiarchis area of Cholomontas mountain, Chalkidiki prefecture, Northern Greece (40°23'N, 23°28'E), which is located in the *Quercion confertae* subzone of the *Quercetalia pubescentis* (sub-Mediterranean) zone. The elevation is about 760–870 m and the climate is characterized as sub-humid Mediterranean, (mean annual air temperature 11.1 °C and annual rainfall 767 mm). The research area is situated in an oak forest (*Quercus frainetto* Ten.), interposed with fragments of natural grasslands. In the area, 1200 goats and 900 sheep grazed continuously for 7 months per year, except for the winter months (Taxiarchis Forest Service Station, 2013). The grazing intensity ranged between no-grazed (Forage Utilization Percentage = 0%), and heavy grazed (FUP > 50%) in the study area (Table 1) (Taxiarchis Forest Service Station, 2013).

The collection sites, with a size ranging from 1 to 2 ha were located in similar elevation, but presented different exposure as they were three Southern and five Northwestern (Table 1). The geographical distances

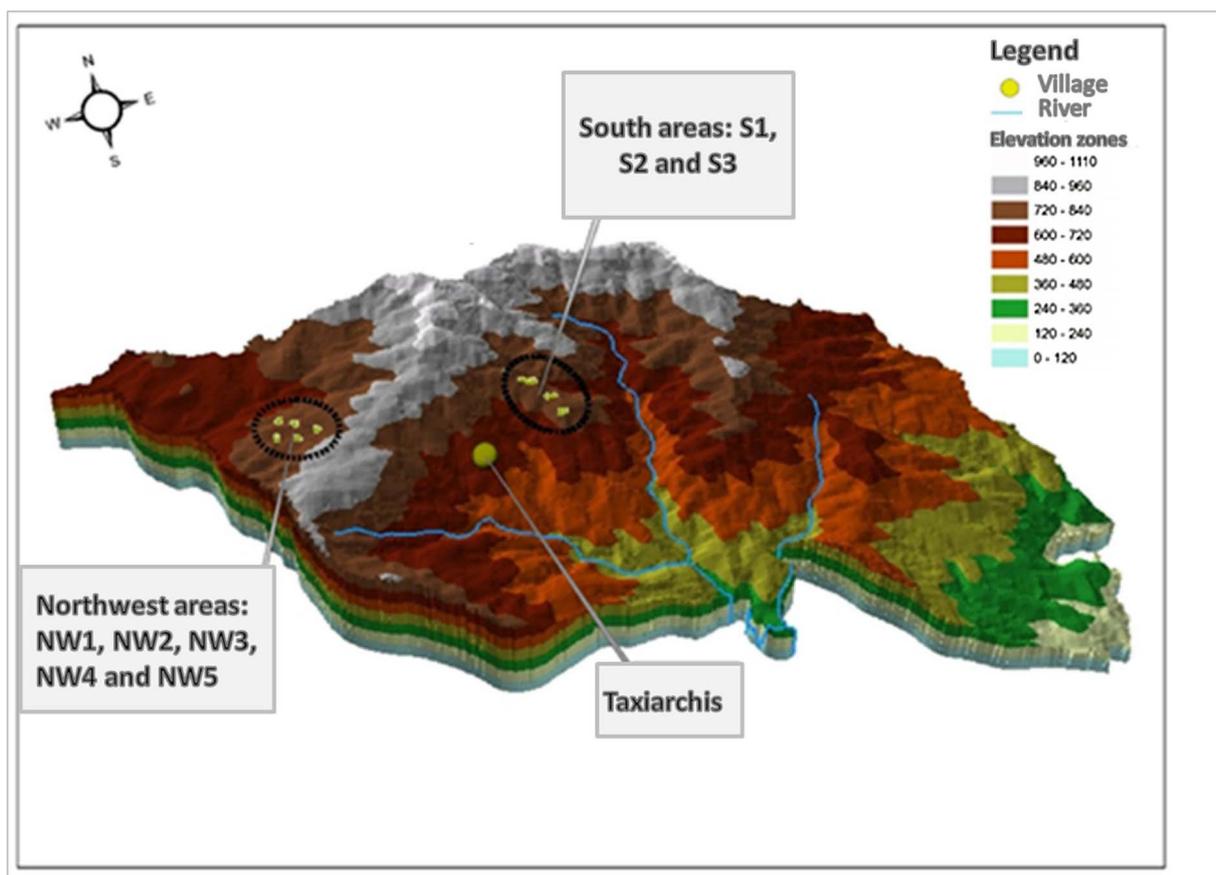


Fig. 1. Map of Southern and Northwestern sites sampled in the Taxiarchis area of Cholomontas mountain, Chalkidiki, Greece.

of the collected sites were between 0.2 and 4 km (Fig. 1). A total of 100 plants at the flowering stage were randomly collected in July 2013. Briefly, the number of individuals per population studied consisted from 10 to 20 (100 individuals in total). The sample sizes per site are presented in detail in Table 1.

The line-point method (Cook and Stubbendieck, 1986) was applied for the estimation of plant cover and the calculation of floristic composition (Table 2). Six transects of 25 m length at each site, with contacts every 25 cm were used along the contour lines at each site in June of 2011. The recorded plant species were categorized in Graminoids (G), Legumes (L), Forbs (F) and Woody (W) species. The Past (Version 3) software (Hammer et al., 2001) was used for the calculation of species richness, species evenness, Shannon Index (I_p) and Morisita-

Horn abundance index. The latter was employed for the comparison of species composition between the studied sites. Soil samples were collected from each site and analyzed as described by Parissi et al. (2014).

2.2. DNA isolation and PCR amplification

The procedure described by Doyle and Doyle (1987) was used for the isolation of total genomic DNA. The amount of DNA was quantified by a UV-spectrophotometer (Eppendorf Biophotometer, Hamburg, Germany) and then the samples were diluted to a 20 ng/ μ L working concentration.

To study inter-simple sequence repeats (ISSRs) eight oligonucleotide primers complementary to simple sequence repeats (UBC807, UBC811,

Table 2

Floristic composition, floristic diversity and the genetic diversity of *T.sibthorpii* at the four souths (S) and the four northwest (NW) sites.

Sites	Plant Cover (%)	Floristic composition (%)					Floristic diversity			Genetic diversity	
		G*	F	L	Th	W	Plant species number	Plant species evenness	Plant species I_p	H_E	I_G
S1	87	29	27	28	16	0	17	0.7	2.4	0.213	0,326
S2	86	31	39	11	10	9	24	0.9	3.0	0.227	0,346
S3	93	34	46	16	3	1	18	0.8	2.6	0.233	0,361
S	89a	32b*	39a	18a	9a	2a	19a	0.7a	2.6a	0.224	0.344
NW1	87	54	24	0.3	20	2	13	0.6	2.1	0.213	0,321
NW2	86	48	28	8	7	5	16	0.7	2.3	0.203	0,311
NW3	88	45	18	0	11	25	11	0.5	1.7	0.245	0,377
NW4	92	58	22	19	1	0	10	0.5	1.6	0.209	0,318
NW5	92	64	14	10	13	0.3	14	0.5	1.9	0.212	0,329
NW	89a	54a	21b	8b	10a	7a	13b	0.6b	1.9b	0.216	0.331

G: Graminoides. F: Forbs, L: Legumes, W: Woody, Th: *Thymus sibthorpii* abundance, I_p : Shannon Index for floristic diversity, I_G : Shannon Index for genetic diversity, H_E : Gene diversity (Nei, 1987).

* Means followed by the same letter in the same column did not significantly differ ($P \geq 0.05$).

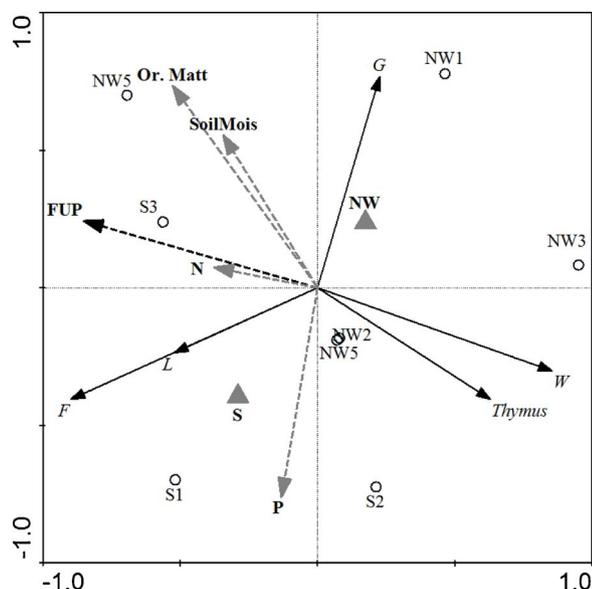


Fig. 2. Biplot of RDA1 showing the position of the following: (i) plant functional groups, (ii) dashed black arrows (significant) and dashed grey arrows (non-significant) soil variables in the four Southern (S) and the five Northwestern (NW) sites. Dummy variables are represented by solid black (significant) and grey (not significant) triangles. Eigenvalues: axis 1 = 0.665, axis 2 = 0.145, axis 3 = 0.100. Cumulative percent variance: axis 1 = 66.5, axis 2 = 81.0, axis 3 = 90.9. S: South, NW: Northwest, FUP: Forage Utilization Percentage, G: Graminoids, F: Forbs, L: Legumes, *Thymus*: *Thymus sibthorpii*, W: Woody.

UBC814, UBC823, UBC827, UBC834, UBC841, and UBC860) were used for PCR amplification. According to the literature review, the specific ISSR markers was selected due to their high polymorphic for the relative species and to their use in a wide range of plant species. PCR amplifications were performed in a PTC 200 (MJ Research Inc, MA, USA), followed by band scoring through gel electrophoresis as suggested by Xanthopoulou et al. (2015).

2.3. Data analysis

Gene diversity, H_E (Nei, 1987) and Shannon's diversity index (I_G) was used as a measure of within-population genetic variability. Furthermore, the genetic differentiation among populations of *T. sibthorpii* based on ISSRs was estimated according to Nei's (1978) genetic distance. A cluster analysis using an unweighted pair-group method with arithmetic averaging (UPGMA; (Sneath and Sokal, 1973)) was carried out using the software Popgene 1.32 (Yeh et al., 1999). Dendrograms were generated using FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). The genetic structure of the *Thymus sibthorpii* populations was analyzed by performing principal coordinate

analysis (PCoA) using GenALEX v. 6.5b5 (Peakall and Smouse, 2006) based on standardized covariance of genetic distance for dominant markers. AMOVA and the individual contributions of slopes exposure and sites on genotypic variability were assessed using GenALEX ver.6.5b5 (Peakall and Smouse, 2006), with sites nested within aspect. The significance was tested by 9999 random permutations, followed by sequential Bonferroni correction.

Correlations of within-population gene diversity (H_E) of *T. sibthorpii*, with species richness and species evenness at each site were tested by Pearson correlation coefficient using the XLSTAT 2014 software (Addinsoft, Paris, France). Additionally, correlations of genetic distances between pairs of populations with the corresponding geographical distances and Moresita-Horn index were also investigated by Mantel test according to Odat et al. (2004).

Analysis of variance (ANOVA) of data was performed using the IBM SPSS Statistics 23 software (SPSS Inc., Chicago, IL, USA) in order to

detect the effect of slopes exposure (Northwestern vs Southern) on species composition and diversity indices. The Least Significant Difference (LSD) at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

The linear method was the appropriate canonical ordination as the gradient of the 1st axis in Detrended Correspondence Analysis (DCA) was < 3 standard deviation units (SD units) (Ter Braak and Smilauer, 2002). Three Redundancy Analyses (RDAs) were carried out as follows: 1) RDA1 for the ordination of the plant functional groups composition, 2) RDA2 for floristic diversity by Shannon's index (I_F) and 3) RDA3 for genetic diversity by gene diversity (H_E) constrained by 7 explanatory variables (Table 1). The slope exposure in RDAs was included in the analyses as a dummy variable. To achieve multivariate normality, all data were logarithmically transformed, except for N of soil features before analysis. The automatic forward selection procedure using the Monte Carlo test with 999 permutations was applied in order to test the significance of the explanatory variables. The multivariate analyses were performed by CANOCO v4.5 for Windows (Ter Braak and Smilauer, 2002).

3. Results

3.1. Floristic composition and diversity of the studied sites

The floristic composition was found to differ significantly between the Southern and Northwestern studied sites. The percentage of graminoids on average was significantly lower at the Southern sites; inversely the percentage of legumes and forbs were significantly higher (Table 2). On the other hand, the percentage of woody species including *T. sibthorpii* did not significantly differ between the Southern and Northwestern sites. Amongst the seven explanatory variables included in the RDA1 (Fig. 2), only the FUP (F -value 6.14, $P = 0.008$) was significant, explaining the 93% of the total variance. As the FUP increased, the percentage of graminoids and woody species including *T. sibthorpii* tend to increase and decrease respectively, mainly in Northwestern sites, while legumes and forbs tend to increase mainly in Southern sites (Fig. 2). The highest value of the Morisita-Horn index (0.68) (data not shown), indicating similarity in floristic composition was recorded between the NW4 and NW5 sites that bared the lowest geographic distance (0.20 km). Inversely, the lowest value of the Morisita-Horn index (0.03) was recorded between the S3 and NW3 sites, being the sites with the highest and lowest FUP respectively.

Moreover, the floristic diversity in terms of species richness, evenness and Shannon index (I_F) was significantly higher at the Southern sites compared to the Northwestern ones (Table 2). Amongst the seven explanatory variables included in the RDA2 (Fig. 3), only the exposure (F -value 7.37, $P = 0.003$) was significant, explaining 83% of the total variance of Shannon index (I_F). According to the RDA2, the Shannon index (I_F) increased at the Southern sites (Fig. 3).

3.2. Genetic diversity estimated by ISSR markers within and between populations

The 8 selected ISSR primers generated 218 loci, of which 71% were polymorphic, while 29% were monomorphic within or between populations. The gene diversity (H_E) and Shannon index (I_G) within the studied populations of *T. sibthorpii* ranged from 0.203 to 0.245 and from 0.311 to 0.377, respectively (Table 2).

The AMOVA (Table 3) revealed that 88% of the total genetic variation resulted from differences within populations. Moreover, genetic differentiation was detected between exposures (i.e. Southern vs. Northwestern sites), as well as among the populations in each exposure (AMOVA, $P < 0.001$) although in both cases was very low, 4% and 9%, respectively (Table 3). Within-population genetic diversity of *T. sibthorpii* did not significantly differed (pooled t -test: $P > 0.2$) between Southern populations (mean 0.224, SD 0.012) and Northwestern ones

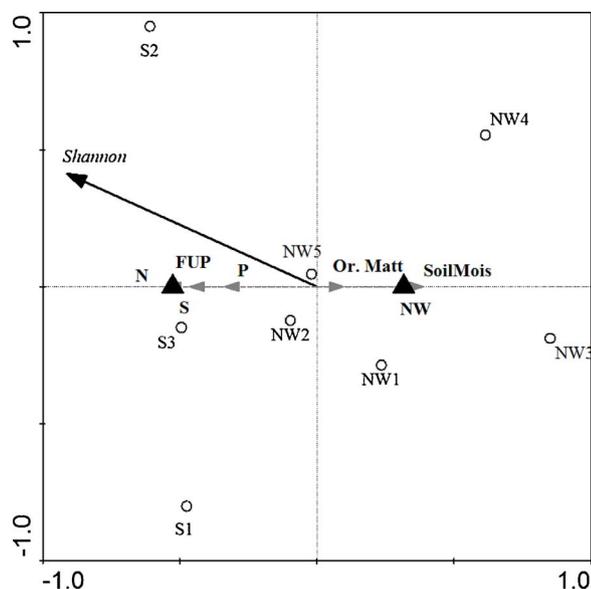


Fig. 3. Biplot from RDA2 showing the position of the following: (i) floristic Shannon Index (I_F), (ii) dashed black arrows (significant) and dashed grey arrows (non-significant) soil features variables in the three Southern (S) and the five Northwestern (NW) sites. Dummy variables are represented by solid black (significant) and grey (not significant) triangles. Eigenvalues: axis 1 = 0.831, axis 2 = 0.169. Cumulative percentage variance: axis 1 = 83.1, axis 2 = 100. S: South, NW: Northwest, FUP: Forage Utilization Percentage, G: Graminoids, F: Forbs, L: Legumes, *Thymus*: *Thymus sibthorpii*, W: Woody.

Table 3

Analysis of molecular variance (AMOVA) for 100 individuals of 8 *Thymus sibthorpii* populations.

Source of variation	d.f.	Sum of squares	Variance	Total%	P-value
Between Populations	7	675.650	4.863	12%	0.117
Within Populations	92	3382.950	36.771	88%	$p < 0.001$
Total	99	4058.600	41.635	100%	
Nested analysis					
Between habitat types (S/NW)	1	171.817	1.633	4%	0.132
Between population/habitat	6	503.833	3.952	9%	$p < 0.001$
Within Populations	92	3382.950	36.771	87%	
Total	99	4058.600	42.356	100%	

(mean 0.216, SD 0.013), being slightly higher in the first.

Nei's genetic distance (Nei, 1973) among *T. sibthorpii* populations ranged between 73.97 (NW1–NW2 populations) and 96.04 (S2–NW4 populations), while the UPGMA dendrogram clustered the populations in two main groups (Fig. 4A). The NW1 and NW2 from geographically proximal sites clustered in the first group. The second group was further separated in two main subgroups where the southern sites S1 and S2 were grouped together. Individual plants from NW1 and NW2 (purple group) as well as S3 (brown group) were grouped together (Fig. 4B). Inversely, individuals of NW3, NW4 and NW5 were not clustered together (Fig. 4B). According to the PCA, the sites with relatively high FUP values tended to locate on the positive side of Coord. 1. (Fig. 5).

Finally, genetic distances were not significantly correlated either with the corresponding geographical distances (Mantel test; $r = 0.302$, $P = 0.48$) (Table 4), or with the Morisita-Horn index (Mandel's $r = 0.280$, $P = 0.51$).

3.3. Correlation between genetic diversity and local plant community

Species richness and species evenness of the plant communities were not significantly correlated to either within population Nei's gene

diversity (H_E) (Pearson's $r = 0.12$; $P = 0.77$, and $r = 0.11$; $P = 0.77$ for richness and evenness respectively), or between population, genetic distances (Mantel's $r = -0.36$, $P = 0.37$ and $r = -0.67$, $P = 0.56$ for richness and evenness respectively). Amongst the seven explanatory variables included in RDA3 (Fig. 6), the N soil content (F -value 9.44, $P = 0.04$) and soil moisture (F -value 9.49, $P = 0.05$) were significant, explaining 99% of the total variance in H_E . The gene diversity (H_E) of *T. sibthorpii* based on ISSR molecular markers generally was higher in areas with higher soil N content and soil moisture (Fig. 6).

4. Discussion

4.1. Floristic composition and diversity of the studied sites

The floristic composition was differentiated between Southern and Northwestern facing sites, despite the sort distance separating them. The graminoid species were predominant in the Northwestern sites, while the legumes and forbs in the Southern ones. However, the abundance of *T. sibthorpii* was similar in both exposures. According to the results obtained by the present study the substantial driver for the differentiation in these mountainous sub-humid natural grasslands was grazing intensity in terms of forage utilization percentage. Grazing intensity explained much more variation in floristic composition than exposure and soil characteristics. Similarly, Allred et al. (2012) reported that plant composition was depended on herbivore species and grazing intensity in semiarid grasslands of the US. On the contrary, Marini et al. (2007) referred that topography was the main determinant for floristic composition in alpine semi-natural grasslands.

The effect of grazing was not consistent in both exposures. Grazing favored the graminoids, especially the tall grasses, such as *Agrostis* sp. and *Holcus lanatus* in the Northwestern sites, while the legumes and forbs were favored in the Southern ones. Usually, the competitive tall grasses are more abundant at high grazing intensities at the favorable sites, as the Northwestern in the present study, that characterized from relatively high resource availability (Cingolani et al., 2005; Dumont et al., 2009). Similar to these results, Lkhagva et al. (2013) suggested that plant communities under grazing in the dry and nutrient poor south-facing steppe shifted to dominance by short and prostrate forb species. These results support the general assumption that spatial (i.e. topography) productivity influences the changes in floristic composition, which is generated by grazing intensity (Milchunas and Lauenroth, 1993; Osem et al., 2004).

The abundance of woody species including *T. sibthorpii* decreased as the grazing intensity increased. This is expected for areas where goats graze. Goats prefer woody to herbaceous species, as their diet consists mainly of lignified species (Aharon et al., 2007; El Aich et al., 2007). This browsing effect on woody cover has been also reported in other studies (Dostálek and Frantík, 2008; Allred et al., 2012). Despite the fact that *T. sibthorpii* is a less palatable species, it is consumed by goats mainly during autumn (Torrano and Valderrábano, 2005), when the content of its essential oils is lower compared to spring (Riddle et al., 1996).

The environmental conditions at the different sites related to slopes' exposure were the main determinant for the observed floristic diversity, as they explained more variation than grazing intensity and soil characteristics. Floristic diversity was higher in Southern sites, which are usually more exposed to stressful conditions, than in Northwestern ones. Similar results were reported by Badano et al. (2005) in the Mediterranean-type matorral of central Chile with an annual precipitation of 300–500 mm. On the contrary, Gong et al. (2008) recorded increased species richness in Northern slopes compared to Southern ones in hilly grasslands of Inner Mongolia, China, with an annual precipitation 250–500 mm. It appears that the results of the present study support the theoretical framework which invokes a dominance of competitive interactions (which reduce diversity) in favorable habitats and a dominance of facilitation (that increases diversity) in stressful

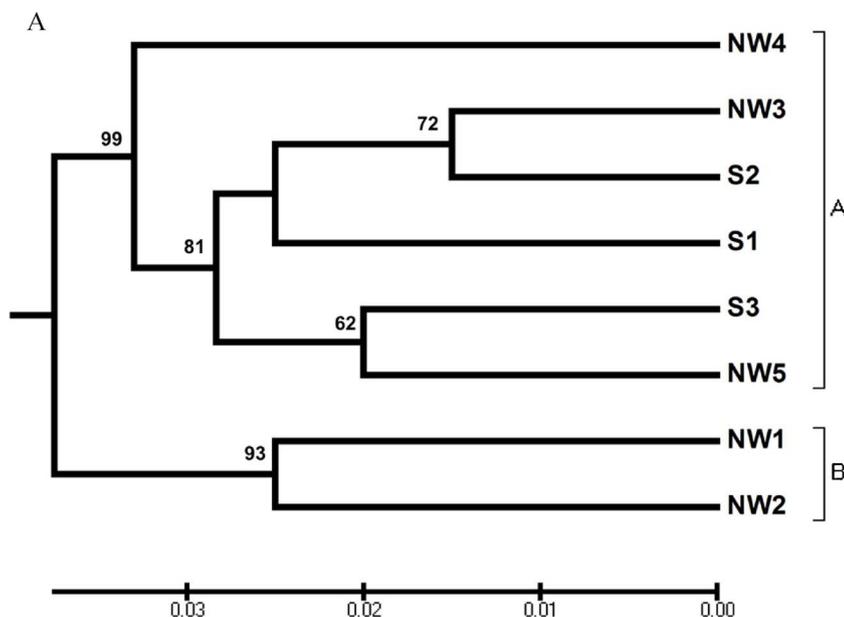
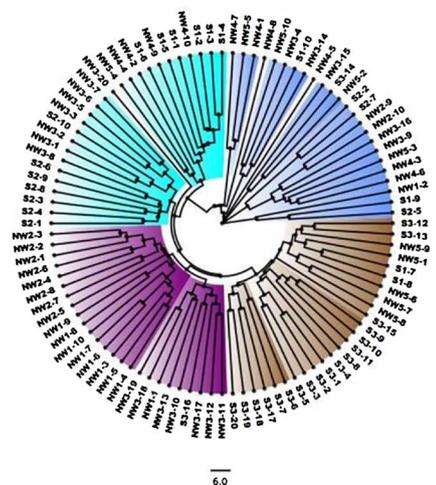


Fig. 4. (A) UPGMA dendrogram based on Nei's genetic distance (Nei, 1987) of *Thymus sibthorpii* populations of Southern (S) and Northwestern (NW) exposures. (B) UPGMA dendrogram based on Nei's genetic distance of *Thymus sibthorpii* individual plants. * Scale bar shows genetic distance. **Numbers on the nodes indicate bootstrap values generated after 1000 replications.

B



habitats (Grime, 2006). Accordingly, the decrease of plant species at the more favorable Northwestern sites was the result of the dominance of few competitors (i.e. *Agrostis* sp., *Holcus lanatus*). On the other hand, at the most stressful Southern sites, the coexistence of many stress-tolerant species of small stature (i.e. *Vulpia* sp., *Cynodon dactylon*, *Trifolium* sp.) was favored.

4.2. Genetic diversity estimated by ISSR markers within and between populations

There is limited information about the genetic diversity of *Thymus* species and in particular *T. sibthorpii*. The present study revealed high polymorphism (71%), which is common for ISSR markers. Similarly, Rahimmalek et al. (2009) reported high polymorphisms using ISSRs for *T. daenensis* subsp. *daenensis*, an endangered endemic species of Iran. High polymorphism was also reported by Trindade et al. (2008) using RAPDs in studies of *T. caespitosus* populations from Azores islands.

A high proportion (88%) of within population ISSR genetic variation was observed. Similarly, Sostaric et al. (2012) showed that most of

the AFLP genetic diversity was recorded within populations of seven *Thymus*, section *Serpyllum* species from Serbia. This is expected for a common, cross-pollinated perennial plant species (Nyblom et al., 1990).

A slight genetic differentiation was observed between exposures (Northwestern vs. Southern), as well as among populations in each exposure. This differentiation was not correlated with geographic distances indicating that even neighbouring populations were differentiated. For instance, the NW1 and NW2 populations were completely differentiated from the NW3–NW5 populations, despite their spatial proximity. The differentiation of populations could be explained by several factors, such as species mating systems, gene flow, genetic isolation, etc., factors that finally shape population structure (Hogbin and Peakall, 1999).

The genetic differentiation of even neighbouring populations likely denotes a low gene flow among them (Hamrick and Godt, 1996). Similar results were reported by Jamali et al. (2012) for *T. capitatus* Hoffm. et Link. and *T. algeriensis* Boiss. et Reut. Generally, *Thymus* species are considered as short distance dispersal species (Thompson and Tarayre, 2000; Tscheulin and Petanidou, 2011). This could be

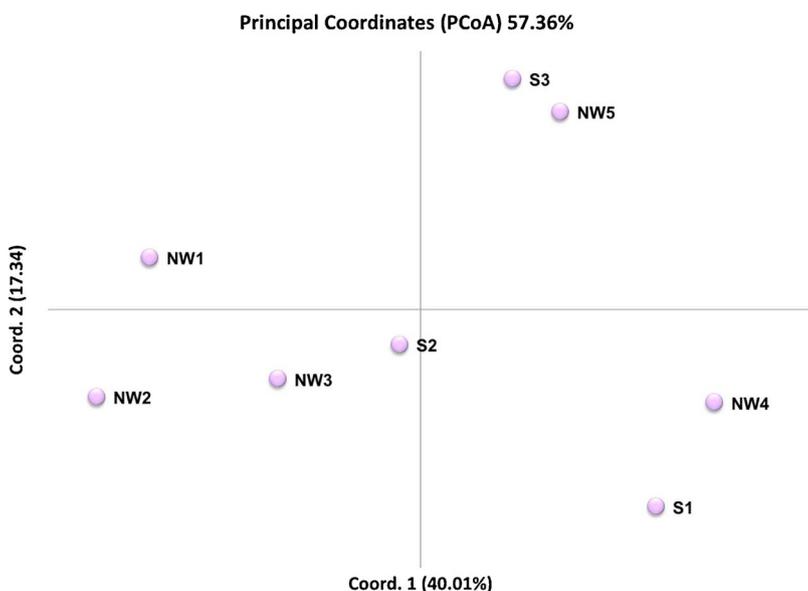


Fig. 5. Principal coordinate analysis (PCoA) of the *Thymus sibthorpii* populations using 218 ISSR markers.

Table 4
Pairwise genetic distances (below diagonal) and geographical distances (in km, above diagonal) between the populations of *Thymus sibthorpii* studied.

Population	S1	S2	S3	NW1	NW2	NW3	NW4	NW5
S1	0	0.35	0.46	3.52	3.71	3.74	3.30	3.50
S2	86.29	0	0.79	3.65	3.88	3.87	3.43	3.61
S3	76.58	82.14	0	3.25	3.43	3.50	3.07	3.27
NW1	82.77	84.82	75.72	0	0.20	0.29	0.30	0.32
NW2	83.90	87.65	79.35	73.97	0	0.33	0.56	0.50
NW3	85.05	84.88	80.18	79.50	79.83	0	0.44	0.28
NW4	83.74	96.04	84.97	93.55	94.29	93.99	0	0.20
NW5	82.28	88.85	75.16	83.25	84.44	87.28	88.58	0

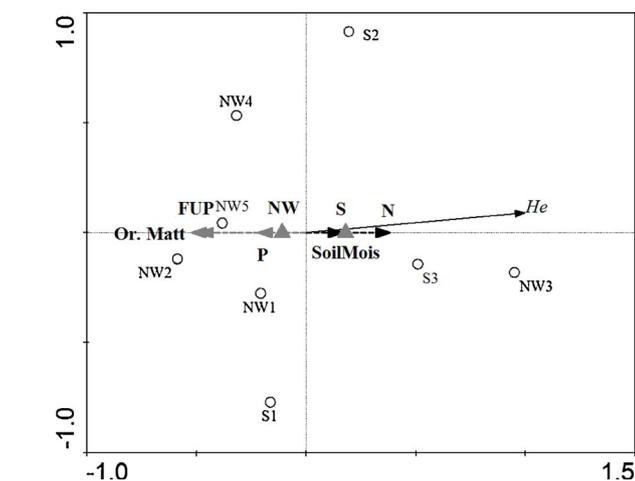


Fig. 6. Biplot of RDA3 showing the position of the following: (i) Nei's gene diversity; (H_E), (ii) dashed black arrows (significant) and dashed grey arrows (non-significant). Dummy variables are represented by solid black (significant) and grey (not significant) triangles. Eigen values: axis 1 = 0.992, axis 2 = 0.008. Cumulative percentage variance: axis 1 = 99.2, axis 2 = 100. S: South, NW: Northwest, FUP: Forage Utilization Percentage.

attributed to the fragmentation of grassland sites and/or the movement and foraging behavior of the pollinators in relation to the mating system of the species. *Thymus* species are characterized by gynodioecy in which female individuals (male-sterile) coexist with hermaphrodite ones in the same population (Dufay and Billard, 2012). The percentage of females in the populations ranged from 5% to 95% with a mean of

60% (Dommée et al., 1978). Females produce the same number of flowers per individual with hermaphrodites, but smaller in size that contain no pollen (Arnan et al., 2014). The *Thymus* species are insect-cross pollinated. According to Arnan et al. (2014) the most frequent pollinators of *T. vulgaris* were bee species (*Bombus terrestris*, *Apis mellifera*, *Lasioglossum* spp. and *Hylaeus hyalinatus*) and the composition of the pollinators were similar for the females and the hermaphrodites. It has to be noticed that the less differentiated populations was the NW3, NW4 and NW5 at sites that beehives usually are placed (Taxiarchis Forest Service Station, 2013).

4.3. Genetic diversity in relation to local plant community composition and diversity

The genetic diversity of *T. sibthorpii* was increased in the more favourable sites that presented higher soil moisture and N content regardless of the slope exposure. This result could not be attributed to the higher population size of *T. sibthorpii*, as the abundance of the species was not affected by soil moisture and N content. It could potentially be explained by resource competition among species (Silvertown et al., 2009). The soil moisture (Donkor et al., 2002) and N content (Yates et al., 2000) increased as grazing intensity increased in the mountainous grasslands. In this respect, grazing appeared to have an indirect positive effect on genetic diversity.

On the other hand, the species' diversity in the present study was correlated with slope exposure and was found higher in the more stressful Southern sites. This result suggests that species' diversity and genetic diversity of *T. sibthorpii* respond in opposite directions to the favorable growing conditions with high resource availability. Species' diversity decreased while genetic diversity of *T. sibthorpii* increased under favorable growing conditions with high resource availability. Similarly, Silvertown et al. (2009) indicated that the genetic diversity of *Anthoxanthum odoratum* increased and the number of associated species decreased by adding resources (N, P, K, Mg) to experimental plots. This response in opposite directions of species diversity and genetic diversity of *T. sibthorpii* suggests a potential negative correlation between them. However, species diversity (richness and evenness) was not correlated with genetic diversity of *T. sibthorpii*, either within or between populations. Probably, the relationship between species diversity and genetic diversity of *T. sibthorpii* in the present study was not defined only by the availability of the resources, but also by other factors e.g. grazing. These results suggest that the relationship between species and genetic diversity is indirect and shaped by the effect of habitat

characteristics on both species diversity and genetic diversity, as hypothesized by Odat et al. (2010).

5. Conclusions

The populations of *T. sibthorpii* are well adapted to both Northwestern and Southern exposures and not affected by the plant community structure in the mountainous grasslands studied. Grazing is a main determinant of species composition in the area, in fact reducing the *T. sibthorpii* abundance. On the other hand, environmental conditions appear to be more important factors for both floristic and genetic diversity. Grazing intensity probably favors indirectly the genetic diversity of *T. sibthorpii*, which appears to increase under favorable growing conditions. This study has shown an absence of correlation between species diversity and genetic diversity in *T. sibthorpii*. This result suggests that from the conservation viewpoint there is not conflict between species diversity and genetic diversity and both could be promoted. However, further research is needed in order to detect how the genetic diversity of *T. sibthorpii* and the effect of environmental factors are related with the quantity and quality of its' essential oil.

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