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TITOLO TESI

Relationships between plant diversity and environmental heterogeneity in rupicolous grasslands on gypsum. The case study of *Alysso-Sedion albi* (Habitat 6110).

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1. Introduction

Plant communities on weathered rock and outcrops are characterized by high values in species richness (Dengler 2006) and often persist on small and fragmented surfaces. Their management and conservation need grounded knowledge about processes and relationships between biotic and abiotic factors both in space and time.

Rocky outcrops are very restrictive habitats for vegetation due to predominance of shallow soils, low water and nutrient retention, high solar radiation, and strong winds (Meyer and Garcia-Moya 1989, 1992). Furthermore, in such environments plant processes strongly depend on highly unpredictable water inputs (Noy-Meir 1973; Westoby 1979, 1980). The establishment of new plant cohorts mainly occurs after a succession of favorable climatic events (Gutterman 1993). Suitable conditions for one particular species or life form, however, may not eventually be favorable for others, resulting in differential temporal patterns of plant recruitment among species or life forms both between seasons and years (Milton 1995; Bisigato 2004).

Substrate characteristics and their variation strongly affects vegetation by regulating both water, nutrient availability (Westman 1981, Kosmas et al., 2000, Raynaud and Leadley 2004, Pueyo 2007) and conditioning establishment and development of plants (Escudero 1996, Escudero et al., 1999, Medina 2006). In addition, substrate heterogeneity may become a determinant factor in maintaining plant diversity (Grime 1979, Tilman and Pacala 1993, Maestre 2003, Bestelmeyer 2006) and the understanding of its relationships with vegetation cover is, then, basic for conservation purpose (Kuntz 2006, Pueyo 2007). High values in plant diversity may be due to the multitude of spatial-temporal micro-niches providing suitable habitats for species with very divergent ecological requirements. Furthermore, the high spatio-temporal heterogeneity prevents any one species from playing off its competitive superiority (Lundholm and Larson 2003). However, essential resources for plant growth are spatially heterogeneous at scales thus co-occurring individuals may find functionally different conditions at very small spatial scales - <1 m (Bell and Lechowicz 1991, Jackson and Caldwell 1993, Ryel et al. 1996, Kleb and Wilson 1999, Fitter et al. 2000).

These systems have been intensively studied with respect to their community composition (Krahulec and van der Maarel 1986, van der Maarel 1988, Poschlod et al. 1996, Dengler and Löbel 2006, 2007) and dynamics (Partel and Zobel 1995, Kalamees and Zobel 1999, Löbel 2006, Lundholm and Larson 2003). Few studies have examined the relationships between heterogeneity and plant diversity at small scales, in particular in poor-nutrient and low productive environment (Shmida and Wilson 1985, Lundholm 2003). At the same time, the detection of seasonal species

occurrence under different micro-environmental conditions has been identified for perennial grasses (Bertiller 1992, Aguiar and Sala 1999, Bisigato 2004) and shrubs (Franco Pizaña et al. 1996, Jurena and Van Auken 2003) in various arid ecosystems but is lacking in rupicolous plant communities and in particular on gypsum outcrops (Pueyo 2007). In Italy, the most important community inhabiting this particular rocky substrate belong to "Rupicolous calcareous or basophilic grasslands of *the Alysso-Sedion albi*" habitat (6110*, 92/43 CEE).

In order to assess change in plant diversity pattern both in space and time on rocky outcrops in northern Apennine, two different approaches were employed in the present study: diachronic and synchronic.

The diachronic survey aims to investigate the seasonal patterns of plant diversity by images analysis techniques integrated with field data and considering also seasonal climatic trend, the substrate quality and its variation in time. Ground Based Photography (GBP) provides high-resolution images in a low-cost approach by using photographs taken at a specific site to monitor conditions or changes (Bennett et al. 2000, Louhaichi et al. 2001, Richardson et al. 2001, Booth 2004, Booth et al. 2005). In particular, it allows the recording of a complex spatial pattern of vegetation on a very detailed scale which can be compared to others both spatially and temporally (Elzinga et al., 2001; Luscier, 2006; Laliberte, 2007). However, most of this study carried out estimation only for total vegetation cover of the plot (Laliberte 2007) without considering change in substrate. For this reason an attempt was made to produce a spatio-temporal model which considered both these factors.

The purpose of the further, synchronic sampling was to describe plant diversity pattern as a function of the environmental heterogeneity meaning in substrate typologies, soil depth and topographic features. It expects to find evidences according to those pointed out by Löbel (2006), Dengler (2006) and Lundhom and Dengler (2007) for the same habitat in Central and Northern Europe. This authors observed a positive relationship with environmental heterogeneity, soil depth and bryophyte mat. Nevertheless the surveyed sites differ from the northern ones in rock composition (i.e. presence of gypsum instead of limestone), habitat extension (which is more restricted in Italy), and climatic trend to latitude gap.

2. Study area

2.1. General features

2.1.1. Geology

The Northern Apennines belonging to the Emilia Romagna administrative region have complex geological history that brought to a various set of rocky substrates, soils and therefore, vegetation. The present study interested a priority pioneer habitat growing up on the rocky outcrops of a peculiar formation constituted by the Messinian Gypsum - Sulphurous Bank, that differs from the Triassic one characterizing the north-west Apennines. The so-called Gypsum vein, which crossed lengthwise the Apennines from Rimini to Zola Predosa, originated following the *messinian saltiness crisis* due to the isolation of Mediterranean Sea from the oceans that caused its almost entire drain with the creation of many evaportic formations. On the surface the formation display a high number of isolated and relatively small outcrops that represent, overall, just shy of 1% of all the regional territory. Nevertheless Apennine Messinian Gypsum bank forms one of the main karstic formations in Italy and Europe, after Sicilian and Spanish ones.
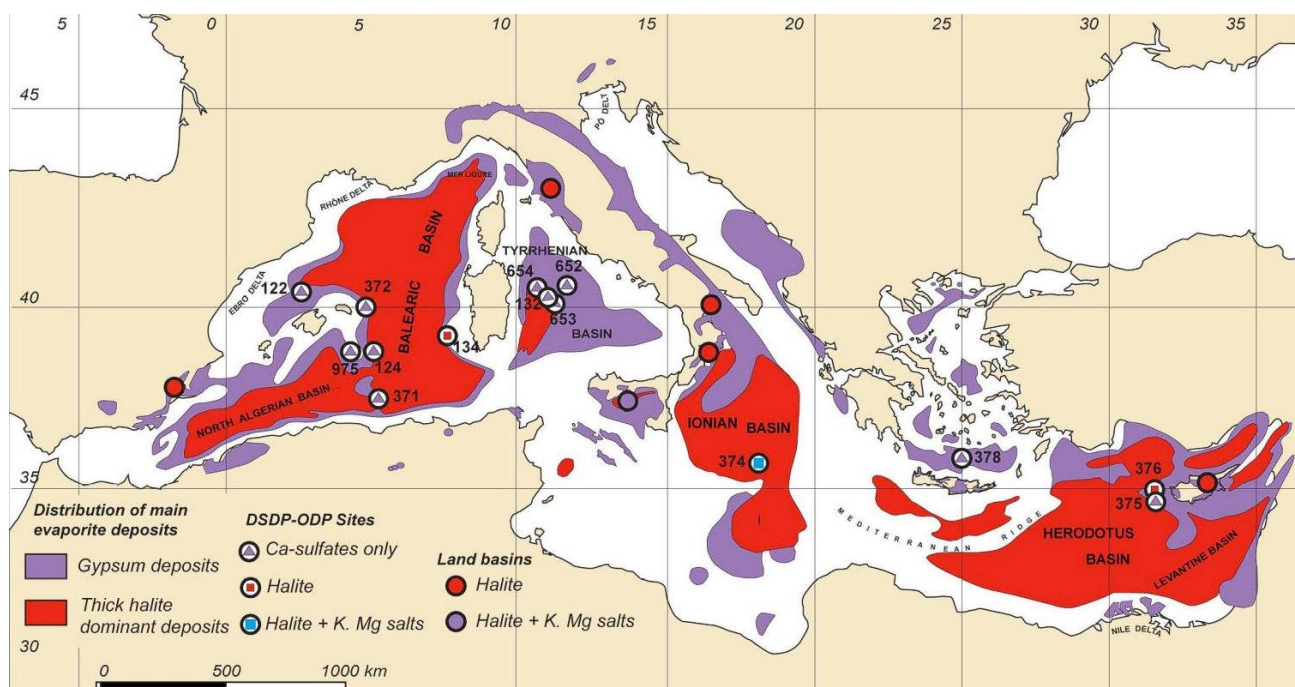

Figure 2.1. Distribution of evaporite deposits in Mediterranean pond. Gypsum deposits are in violet.

The high solubility of the gypsum rock produced in the karstic landscape a great number of interesting morphological features recognizable both in large and very fine scale. These morphologies associated with many climatic and topographic situations differentiate karstic

Figure 2.2 Meso karstic feature: the candle's hole in Miserazzano.

landscape from calcareous ones. One of the principal geomorphological large and meso features recognizable in gyspum formations are the sinkholes. These natural depressions are originated by the collapse of subterranean caves or erosion of gypsum rocks by water streams which generates many environmental niches. Generally the bottom of a sinkhole presents a thermal inversion with fewer temperatures and more wetness; otherwise the upper edges are often characterized by rocky outcrops with thin layers of soil. Sinkholes can occupy many square kilometers or few meters. Other typical small karstic features are the *karren,* a system of very small cracks and bowls generated by the chemical action of water on the gypsum. Gypsum rock has a long history of human exploitation, particularly near the principal cities, like Bologna, as building material. However, during the last century protected areas were created in Italy with the purpose of preserving gypsum formations through the institution of some Sites of Community Importance (SCI) within the Natura 2000 network (Habitat Directive 92/43 CEE). The present work focuses on the outcrops relative Bolognese's Appenine belonging to two SCI.

2.1.2. Bioclimate

The climatic profile that characterize rocky outcrops of northern Apennine follows a continental – meso-mediterranean trend with cold winters and warm summers with mean annual temperature of 15.7 °C. The mean temperature value of the coolest month (January) is 4.2 °C while the mean temperature value of the warmest month (July) is 24.9 °C. The lowest temperature value of the sampling period was -11.1 °C. Annual rainfall averages 888.8 mm, October being the wettest month with an average of 88.4 mm and July the driest with 45.6 mm (data from the climatic station of Bologna and Zola Predosa, ARPA Emilia Romagna). From December to February usually there is the possibility of one or more frost periods. Anyway, Gypsum crags have their own microclimate individuality (Ferrari, 1974). Microclimate conditions depend on slope aspect, plant species covering and on substrate characteristics and evolution degree. Slopes with a southern aspect receive a higher solar radiation along the whole year and then the temperatures are always higher than other areas. During the summer period these slopes can be subjected to an intense warming because solar rays can came up with a 90° inclination value. In addition, Pullega (1992) showed that on solar radiated bare gypsum rock (because of its heat capacity) temperatures are 3-4 °C

higher than the air at 1-2 m above the ground. Because of sediments flushed away no much water can be held and it flaws away too. This lead to both unstructured vegetation covering presence and extended temperatures range.
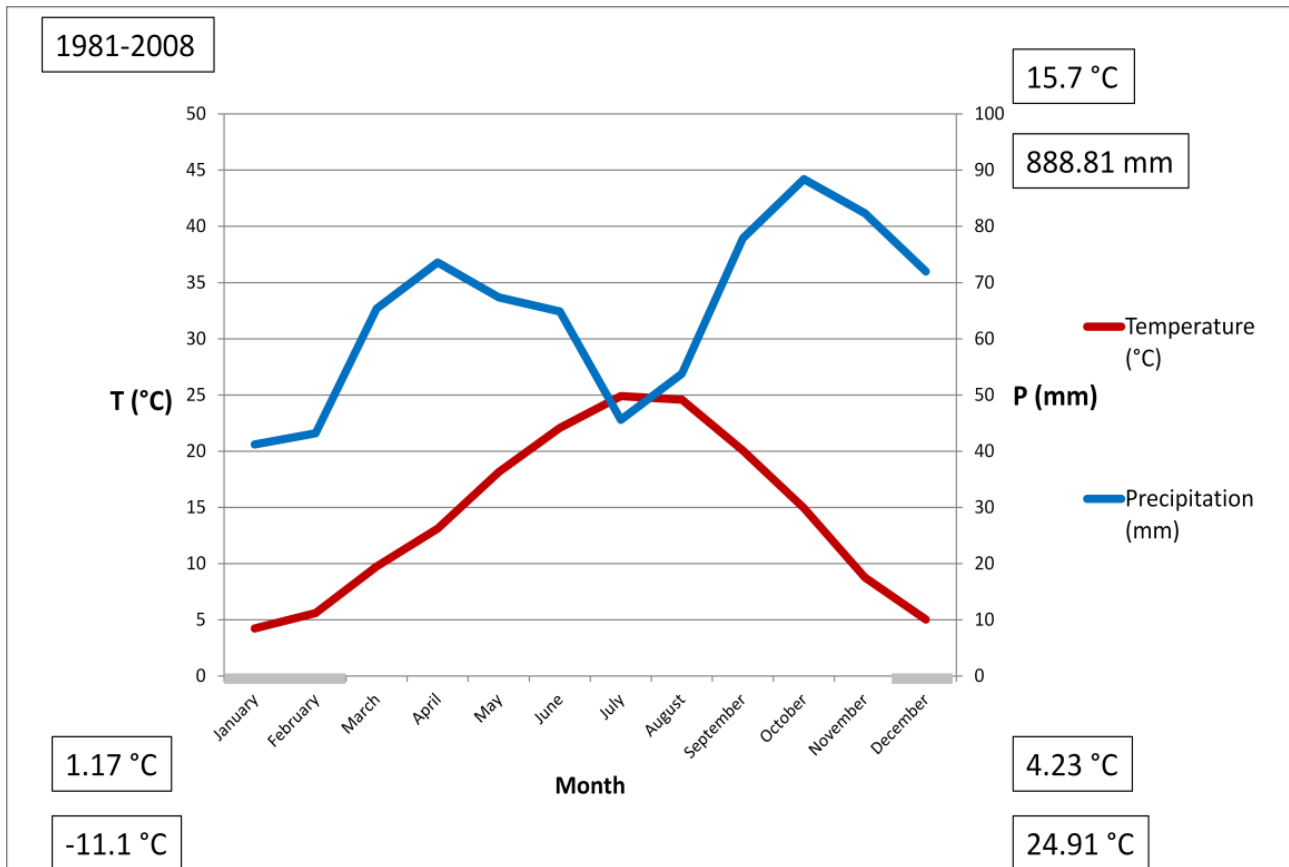

Figure 2.3 Ombrothermic diagram of Bolognese's gypsum outcrop. Altitude 210m

2.1.4. *Vegetation: the* "Rupicolous calcareous or basophilic grasslands of *the Alysso-Sedion albi*"

Gypsum outcrops are characterized by good stability of environmental conditions but with strong constraining factor for vegetation suitability. Physic, chemical and topographical gypsum features influence directly and indirectly plant colonization and their spatial patterns (Pueyo 2007, Noy-Meier 1973; Dodd and Lauenroth 1997). The vegetation inhabiting such hard-living places is, therefore, formed by plants specifically adapted like "*xerothermophile pioneer communities dominated by annuals and succulents*" (Oberdorfer & Müller in Müller 1961). These communities represent the vegetation reference for a priority habitat of European community: the "Rupicolous calcareous or basophilic grasslands of *the Alysso-Sedion albi*" moreover codified as habitat 6110*. This vegetation is constituted by discontinuous and scatter patches, depending on the quality of the local substrate. Biondi et al. (2009) defined it as "patchy grassland [...] vegetation, with calcareous

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mosses and lichens, which is present between meso-Mediterranean and lower super-temperate plane and locally up to subalpine one. [...]". The physiognomy species combination, reported by the Italian Interpretation Manual is shown in table 2.1:

Table 2.1 Species of the *Alysso-Sedion albi* habitat. Biondi et al. (2009).

Species	Life-form	Species	Life-form
Alyssum alyssoides	T	*Poa molinerii*	H
Alyssum montanum	Ch	*Ptychotis saxifraga*	H
Arabis auriculata	H	*Saxifraga tridactylites*	T
Bombycilaena erecta	T	*Sedum acre*	Ch
Catapodium rigidum	T	*Sedum album*	Ch
Cerastium brachypetalum	T	*Sedum dasyphyllum*	Ch
Cerastium glutinosum	T	*Sedum hispanicum*	T
Cerastium pumilum	T	*Sedum montanum*	Ch
Cerastium semidecandrum	T	*Sedum rupestre*	Ch
Cladonia pyxidata	(L.)	*Sedum sexangulare*	Ch
Erophila verna	T	*Sempervivum tectorum*	(group)
Fulgenzia fulgens	(Sw.)	*Teucrium botrys*	T
Geranium molle	H	*Thlaspi perfoliatum*	T
Hornungia petraea	T	*Tortella inclinata*	(R.Hedw.)
Jovibarba spp.	Ch	*Syntrichia ruralis*	(Hedw.)
Melica ciliata	H	*Trifolium scabrum*	T
Minuartia hybrida	T	*Triticum ovatum*	T
Orlaya grandiflora	T	*Valerianella eriocarpa*	T
Peltigera canina	(Weiss)	*Valerianella rimosa*	T
Petrorhagia prolifera	T	*Veronica praecox*	T
Poa badensis	H		

Ferrari et al. (2011) reported as observed species of the habitat in Emilia-Romagna also some species which are not mentioned in "The Emilia-Romagna Manual of Habitat". These are:

- *Sedum dasyphyllum*;
- *Triticum ovatum*;
- *Petrorhagia saxifraga subsp. saxifraga*;

Under a serial point of views, these communities are substantially stable due to extreme and peculiar environmental conditions. However, sometimes it's possible to recognize some still more primitive terms, formed by calcareous moss (*Tortellion* spp.) and lichen (*Toninion coeruleo-nigricantis*) that can be referred to the habitat 8210 (Calcareous rocky slopes with chasmophytic vegetation) (Di Pietro et al 2006). Habitat identification in field is not always easy because of its numerous intersections and promiscuity with other communities (Fig 2.4). Depending on the soil

availability and topographic factors, there are frequent several contact point with vegetation ascribable to xeric grassland of *Festuco-Brometalia* (habitat 6210* - Semi-natural dry grasslands and scrubland facies on calcareous substrates) or to the *Juniperus communis* formations on heaths or calcareous grasslands (habitat 5130). Furthermore not always clear it seems to be the edge with thermophile aspect of the limestone pavements (habitat 8240).

Figure 2.4 Habitat 6110*: as it looks in Romagna (on the left) and in Zola Predosa, Bologna (on the right).

Due to its unexpected high level of diversity, many studies were carried out to describe the vegetation inhabiting habitat 6110*. Among them, in Italy, the main are Macchiati (1888, 1891, 1892), Cobau (1932), Zangheri (1959), Bertolani Marchetti (1960, 1961), Corbetta (1965, 1967), and Ferrari, (1974) and Aleffi and Silenzi (2000), for the bryophytes. However, does not univocally fit in any phytosociological syntax. Biondi et al. (2009) report that in northern Apennines these cenosis had to be referred to *Petrorhagio saxifragae - Sedetum sexangularis* (Venanzoni e Gigante 1999) while, in Emilia-Romagna Apennines, Ferrari (1974) suggested the *Cladonio–Sedetum hispanici* (CORINE code: 34.111) and then Bassi (2007) mentioned also the *Cerastietum pumili* (Oberd. et Th. Müller in Müller 1961) (Corine code: 34.11). *Petrorhagio saxifragae-Sedetum sexangularis* and *Cerastietum pumili* were, however, included in *Alysso alyssoidis-Sedion albi* (oberdorfer & müller in müller 1961, *Sedo-scleranthetalia* br.-bl. 1955 and *Sedo-scleranthetea* br.-bl. 1955 em. th. Müller 1961). The hypothesis about the existence of a strictly gypsophyle vegetation was definitely reject by Zangheri (1959) who set these communities between the generic chalky and xeric vegetation. However, it cannot be excluded the role of gypsum in creating different micro topographic feature that may influence diversity pattern respect to the limestone outcrops.

In Emilia-Romagna region 6110* habitat is present in 44 sites in an overall extension of 803 ha. Within the Region (Bassi, 2007) the 6110* habitat can be observed on:

- Gypsum outcrops;
- Limestone outcrops (eg. in Sassoguidano and on Pietra di Bismantova).

Usually this vegetation is fragmented reaching, sometimes, a few square meters of surface. In Emilia-Romagna, only on the gypsum outcrops is possible to find the widest sites of this habitat. According to Life Gypsum (2010) it is present in several ICSs that preserve also gypsum outcrops (Table 2.2).

Table 2.2. Extention and relative cover percentage of 6110* Habitat in Emilia-Romagna ICSs (Life Gypsum, 2010).

Site	Habitat surface (ha)	Cover for each site (%)
IT4050001 - Gessi Bolognesi, Calanchi dell'Abbadessa	2,2	3
IT4070011 - Vena del Gesso Romagnola	126,34	2
IT4030009 - Gessi Triassici	38,14	2
IT4030017 - Ca' del Vento, Ca' del Lupo, Gessi di Borzano	11,37	1
IT4050027 - Gessi di Monte Rocca, Monte Capra e Tizzano	4,52	2
IT4090001 – Onferno	0,31	3

The present research was carried out in two Important Community Sites that include a broad system of gypsum outcrops in northern Apennine of Emilia-Romagna administrative region: IT 4050001 "Gessi Bolognesi e Calanchi dell'Abbadessa" and IT 4050027 Gessi di Monte Rocca, Monte Capra e Tizzano.

2.2. "Gessi Bolognesi e Calanchi dell'Abbadessa"

In Bologna province gypsum outcrops cover approximately 55 ha (excluding the huge subterranean web of caves and tunnels of almost exclusively zoological and geological interest)   and ranges in elevation from 70 to 400 m a.s.l. with varying slope and exposures. This outcrops are characterized by high fragmentation and small and isolated patches and, although the whole Emilia Romagna's gypsum formation represents only the 1% of all regional surface, it is the second largest gypsum bank in Italy, after Sicilian ones, and one of the most important at European level (Spipola's sinkhole in Bologna is the biggest one in Europe with a diameter of 600 m and a deepness of nearly 100 m) both for geological and biological aspects.

In the park of "Gessi Bolognesi e Calanchi dell'Abbadessa" the largest outcrops, included in the study, is the Miserazzano upland. It is a 5 ha wide karstic area that overlooks Southern Po floodplain around San Lazzaro di Savena and Bologna suburbs and the Savena valley. Miserazzano is included in the karstic territory called Croara, a few square kilometres wide and sited between

Zena stream and Savena River. The Croara territory in general and Miserazzano in particular, lies above one of the most important karstic systems, known as "Spipola - Acqua Fredda" (Forti, 1995). This upland is constellated of small dimension ponors and sinkholes that cause a patchwork of small mounds and depressions with several aspects (Pullega, 1992). The result is a very heterogeneous landscape, which is, also, exacerbated by other medium size karstic features, in particular gypsum unstacked bubbles. On the Miserazzano upland the habitat 6110 covers a surface of about 2 he and it shows a close intersection with many other habitat of European interest such as 6210 (Semi-natural dry grasslands and scrubland facies on calcareous substrates) where there is a thicker bed of developed soil and 8210 (Calcareous rocky slopes with chasmophytic vegetation) on wetter and steep slopes.

Figure 2. 5 View from Miserazzano upland

The outcrops vegetation is also characterized by the presence of Mediterranean and steno-mediterranean species particularly adapted to a warm and dry climate and to high radiation levels. Some of the species that take refuge on this area, such as *Quercus ilex, Phyllirea latifolia* and *Rhamnus alaternus*, are uncommon for this region. Here Ferrari (1974) described the *Cladonio-*

Sedetum hispanici association, stressing for the first time the main importance of mosses and lichens for the colonization processes of this habitat. The characteristic species individuated for this association were *Sedum hispanicum, Bromus sterilis, Bryum bicolor, Barbua convolute, Cladodia convolute and Cladonia pyxidata*. Ferrari (2011) recorded a wide presence of other species like *Erysimum pseudorhaeticum, Catapodium rigidum* and *Sedum sexangulare.* After the institution of the ICS and the Regional Park, the main pressure is represented by the public fruition meaning as foot crushing considering the easy access and the scenic view offer by the relative high position of the upland.

2.3. *"Monte Rocca, Monte Capra and Tizzano"*

The ICS of Monte Rocca, Monte Capra e Tizzano represent the extreme edge of the Bologna's Messinian Gypsum-Sulphurous Bank beyond Reno River. The area interested by the study included the so-called Gessi di Zola extended from Monte Rocca long the arch ending at the Monte Magalotto. In spite of its small extension, few tens of hectares, this site it considered one of the most important in the Region in terms of karstic features composed by many cliffs and small isolated uplands. The small outcrops where the habitat is present are organized in many rocky bulge constituent the top of a group of small elevations. Similarly to Miserazzano upland, Monte Rocca shows a high mix of many habitats linked to xeric and rocky environment (6210 and 8210 *in primis*), but here the small surface and the fragmented disposition make this outcrops more vulnerable to the evolution of vegetation, in particular due to the very close proximity of the *Quercus* spp. and *Carpinus* sp. mesophile wood. The only floristic notes available for this site is represented by the samples of Ferrri (2011) which recorded as main characteristic species: *S. sexangulare, S. hispanicum, S. album, Cerastium pumilum, Bromus sterilis* and *Silene vulgaris*. The site doesn't suffer of particular threat due to human activities and to difficult access caused by both low visibility and the unsafely condition of the outcrops.

3. Materials and methods

Diachronic and synchronic samplings from April 2012 to March 2013 were performed. Habitat detection was helped by a digital map from the EU Life Project "Gypsum" (2011). A 50x50 cm *plot* was used in both samplings such as the sampling unit base, and they are subdivided in a grid of 100 5x5 cm subplots (Fig. 3.1). This plot dimension allowed to reduce within-plot heterogeneity, obtaining a better relationship between substrate and vegetation, and at the same time to sample a representative portion of the community (Wilson *et al.* 2012, Klimes 2001).

Figure 3.1. The plot. A 50x50 cm metal frame subdivided in 100 5x5cm subplot grid.

3.1. Diachronic approach

3.1.1. Sampling design and field data collection

The sample sites were Miserazzano and Mt Castello, in the Northern Apennines near Bologna, at about 200 m asl, and southern exposure. In order to establish a permanent quadrat (150x150 cm) nine adjacent *plots* (50x50 cm) were arranged hereafter and referred to as *macroplot* (Fig 3.2). Two macroplots were placed in the sampling areas of Miserazzano (GB1 and GB2) and two on Mt. Castello (GZ1 and GZ2) (Table 3.1). Each plot was fixed on the gypseous rock by adjustable screws. The macroplot inclination was evaluated by an inclinometer and its correct exposure was evaluated by a compass. Some screws also allowed an accurate identification of the sampling site for the subsequent data collection. Geographical position and elevation were recorded using GPS receiver (Trimble GeoXT) and an altimeter.

Table 3.1. Macroplot data station and sampling temporal steps.

Sampling area		Miserazzano		Monte Castello	
Sampling site		**GB1**	**GB2**	**GZ1**	**GZ2**
Latitude		44°26'45.65" N	44°26'45.49" N	44°27'53.90" N	44°27'53.85' N
Longitude		11°22'26.20" E	11°22'26.71" E	11°13'08.48" E	11°13'08.50" E
Slope		26°	37°	20°	25°
Recording date	**1**	03/04/2012		08/04/2012	
	2	02/05/2012		08/05/2012	
	3	02/06/2012		03/06/2012	
	4	04/07/2012		02/07/2012	
	5	03/10/2012		09/10/2012	
	6	04/11/2012		10/11/2012	
	7	05/12/2012		07/12/2012	
	8	03/02/2013		04/02/2013	
	9	07/03/2013		08/03/2013	

A Nikon 24 mm lens, D80 digital camera, was used to take an image of the plot, hereafter named as photoplot (Fig. 3.3). The camera was located on a tripod in a zenithal position (1.2 m) in order to reduce distortion effects and its relative position in connection with the plot was checked by a spirit level. The shadow effect was minimized by taking the photoplot between 11.00 am and 2.00 pm. Frames were recorded in RAW format. The photoplots allowed to identify plant species and to describe their cover as well as to record the presence of single non-desiccated vascular species.

We referred to Pignatti (1982) for species identification and to Conti et al. (2005) for their actualized nomenclature. Thus the identification was not fully possible, the identification was restricted to the genus. The environmental heterogeneity was recorded at subplot level as a list of different substrata: bryophytes, litter, bare soil, bedrock and gravel (Lundholm & Larson 2003, modified; Table 3.2). Survey was repeated almost every 30 days from April 2012 till late March 2013 (Table 3.1) avoiding for August and September (corresponding to the dry period) and January, because of the snow cover.

Table 3.2 Data collected in the field.

Variable	Details	Scale
Exposure	Exposure (degree) measured at the center of each plot	Macroplot
Slope	Slope (degree) measured at the center of each plot	Macroplot
Vascular species	Census of species	Subplot
Substrate cover	Bedrock, Gravel = rock moveable by hand, Soil = bare soil, Litter = dead vascular plant litter, Bryophytes = bryophyte mat extension (Lundholm & Larson, 2003, modified)	Subplot

3.1.2. Image processing

Photoplots were processed using Photoshop CS 5 (Adobe Systems Software) lens correction filter that fix common lens flaws, such as barrel and pincushion distortion, vignetting, and chromatic aberration. The filter was also used to rotate the plots and solve perspective issue caused by vertical or horizontal camera tilt. The grid on the plot facilitated this operation. Images were loaded into ArcGIS 9.2 (http://www.esri.com) and mosaiced to reconstruct each macroplot. Four different types of layers were derived from image interpretation:

- vascular species cover;
- substrate cover;
- vegetation subplot frequency;
- substrate subplot frequency.

The vascular species and substrate cover layers were obtained by visually interpreting and simultaneously digitalizing the different plant species and substrate types cover with the support of the data collected in the field. Figure 3.2 shows the image processing framework.Subplots frequency layers were carried out by superimposing a Digital Grid Overlay (Corel 1997, Booth 2006, Boot 2005a) which digitally subdivides in 300x300 cells ($0,25$ cm^2 on the ground) the macroplot area and corresponds to a 100 cells subdivision of each subplot.
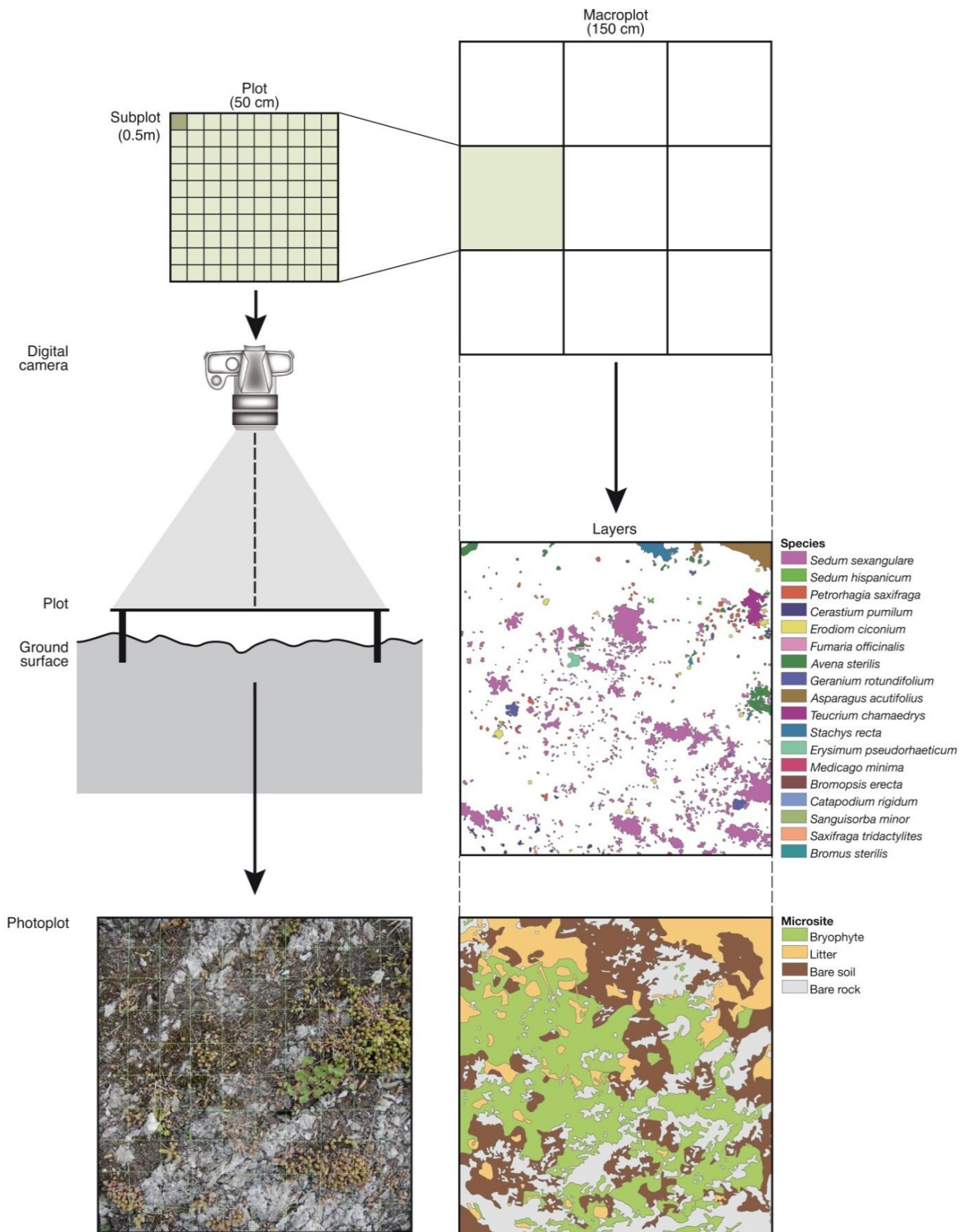
Figure 3.2 Sample design with the 50 cm plots assembled in 150 cm macroplot. Each plot is subdivided into a grid of 5x5 cm (subplots). In the field, a photograph for each plot. Microsite and species cover layers were derived after mosaicing and interpreting the photoplot of each macroplot.

In each cell was recorded the presence/absence of vegetation or of target substrate typology and finally it was calculated the frequency of the variable target in each subplot (Fig. 3.3).
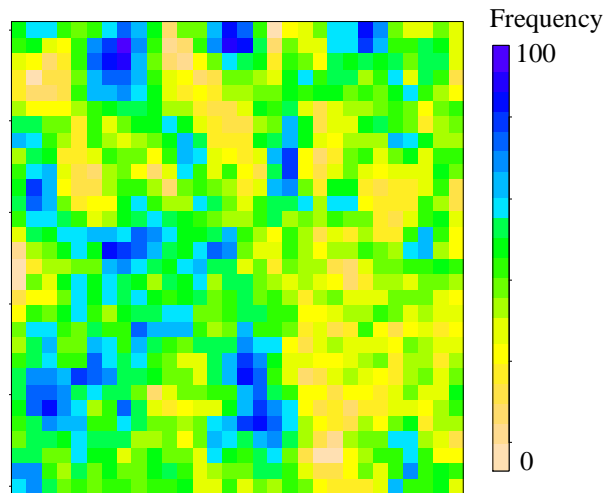

Figure 3.3 Vegetation subplot frequency layer.

3.1.3. Data analysis

The vascular species cover and substrata cover layers were converted into raster datasets (20 pixels size) and, subsequently, converted into ASCII files.

These data allowed by computing of some metrics (Table 3.4) using Fragstat 4.1 (http://www.umass.edu) software both at *class* (species or substrate) and *landscape* level (macroplot) according to McGarigal & Marks (1995) in the following elements: landscape percentage, number of patches, patch richness, Shannon diversity index. The percentage of landscape of each species was evaluated both in absolute and relative terms. The sum of the percentage of landscape of each species gives the total vegetation cover in the macroplot and its complementary to the empty space (*matrix*). Shannon's diversity index (Shannon & Weaver 1949), considering patch covers, was used because of its higher sensitivity to rare patch types than Simpson's diversity. The chi-squared test was applied to verify the effect of the inter-annual period on the variables, and the Wilcoxon paired test was performed to test possible differences between macroplots trends. Species were also grouped and analyzed according with Rankier's Life Forms (1934).

Cluster analysis among macroplots, based on presence/absence of vascular species, was carried out to group macroplots by their similarity degree. The similarity was calculated by the *Jaccard Index* (Jaccard, 1901):

$$IS_j = \frac{c}{a + b + c}$$

Where c is the number of common species, a is the number of unique species to the first macroplot, and b is the number of unique species to the second one.

Changes in species composition between two subsequently temporal steps were detected by using Turnover Index (TI) (Otsus, M. & Zobel, M, 2002 modified):

$$TO = \frac{a + d}{a + d + pr} * 100$$

Where a (appearance) is the number of new species appeared during two consecutive temporal steps (from July to July), d (disappearance) is the number of species disappeared, and pr (persistence) is the number species that endure during the considered period. The index ranging from 0 to 100 with the persistence of all the species at 0 and the total change of community at 100. Turnover was also performed considering the whole period from April 2012 till March 2013 to evaluate possibly change in species pool overall the sampling year.

Correlation between diversity, species turnover and annual climate was performed by using interpolated temperatures and rainfall data available from Settefonti's meteorological station (San Lazzaro di Savena) for the years 2012-2013. Monthly mean temperatures and precipitation values were regressed against Shannon diversity index and turnover index of all macroplots of all temporal steps. In order to test possible differences between macroplots, an ANOVA was performed using the residual values of the response variables of the regressions. Statistical computations were executed using R 3.0.2 software.

An indirect ordination technique (Correspondence Analysis: CA) was carried out in CANOCO 4.5 using the temporal steps as sample and the relative cover (log transformed) of species as variables to easily explore the species temporal pattern in the four macroplots. In the analysis were only considered the species reaching at least the 1% of landscape cover in, at least, one temporal step. A synthetic scheme was built by joining the single species cover over the time with temperature and rainfall diagram, in order to better visualize the single species trend during the sampled year.

In order to investigate the relationship between vegetation coverage and substrate typologies, a generalised linear mixed model (GLMM) is proposed in a hierarchical Bayesian framework.

For modelling occupancy probability the *probit* link function was adopted; then the linear predictor is specified as follows:

$$\Phi^{-1}\left(\pi_{st}\right) = \eta_{st} = \boldsymbol{x}_{st}^{T}\boldsymbol{\beta}_{t} + \alpha_{t} + \theta_{s} + \delta_{st} \qquad s = 1,...,S; \ t = 1,...,T$$

where π_{st} denote the occupancy probability at plot s and time t. The number of cells is denoted as n, S is the number of subplots and T is the number of temporal steps. Parameters $\boldsymbol{\beta}_{t}$ denote time-varying regression coefficients. The model is fitted to data structured on the species and substrate frequencies layer of GB1 macroplot. Basically, the model is intended to capture the relationship between substrates typologies (covariates) and the measured vegetation cover between couples of temporal steps, taking into account temporal dependence as well. The proposed model contains, other than parameters indicating the effect of each substrate typology (i.e. covariate effects), random effects capturing residual spatio-temporal variability unexplained by the dependence upon the covariates (i.e. spatiotemporal random effects).

3.2. Synchronic approach

3.2.1. Sampling design and data collection

Synchronic sampling was carried out in May 2013, when most of the species reached peak in cover, flowering or fruiting. Nineteen plots were randomly located within polygons belonging to the target vegetation individuated on the digital cartography of the habitats. To avoid as much as possible the spatial autocorrelation of data, a minimum distance of 5 meters has been maintained between plots. If a plot fell upon a shrub above 0,5 m in height a new plot was chosen, because of its small dimension. Plots that fell under tree canopy (tree height >1.5 m) were included. The visual cover assessment of total vegetation and each single species was performed separately by summing the cover values of each subplot composing the 10x10 grid: each subplot were subdivided in four quadrant considering each one as 0,25% of subplot cover if occupied by a species. Richness and Shannon's diversity indices (Shannon and Weaver 1949) were calculated for each plot. The phenology (vegetative and flowering) of each species was also sampled following BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) simplified scale (Hack et al., 1992; Meier, 1997). The phenological stage of each species was determined on the average of the stages of at least ten individuals in the macroplot or immediately nearby. Table 3.3 summarizes the variables afferent to the vegetation.

Table 3.3 Considered variables relative to the vegetation.

Diversity variables	Details
Single species cover	Sum of the subplot cover measures
Total species cover	Sum of single species covers
Species richness (S)	Count of all the species in a plot
Species phenological stages	BBCH method (Hack et al. 1992; Meier 1997, simplified):1=germination, 2= leaf development, 3= stem elongation or rosette growth,4=vegetative development, 5= inflorescence emergence, 6= flowering,7= development of fruit, 8= maturity of fruit and seed, 9= Senescence/ beginning of dormancy
Diversity index (H)	Shannon diversity index (Shannon and Weaver, 1949)

Environmental variables collected for each plot were in Table 3.4. Tree layer cover was visually estimated in a 0,5-m radius around the plot. The soil depth for each plot was obtained by inserting a metal pin 0,5 cm in diameter, until resistance was met, and by calculating the mean of the measures at the center of ten fixed subplots. Furthermore the presence of human locally trampling, was also recorded using a presence/absence scale.

Table 3. 4 Environmental variables took into account in the study.

Environmental variables	
Aspect	Degree (°)
Slope	Degree (°)
Substrate cover	Percentage of rock, soil, litter, bryophyte and gravel cover
Substrate richness	Number of substrate types in a plot
Substrate heterogeneity	Shannon diversity index applied on microsite cover
Mean soil depth	Mean depth of soil measured in ten fixed subplot
Shadow	Percentage of shadows measure in a radius of 0,5 m around the plot
Human disturbance	Presence/Absence of pounding (Factor)
Topographical features	Classified in F=flat, CX=convex, CV=concave,I= irregular (Factor)

3.2.2. Data analysis

A species rarefaction curve was used to test the right number of sampled plots to reach the most part of the species in the area (Colwell et al. 2004) by using PAST 2.17c (Øyvind Hammer, 2013). Species were also grouped and analyzed according with Rankier's Life Forms (1934). Separate Kruskal-Wallis test were employed to find possible effects of disturbance, topographic features and substrate richness on biodiversity, vegetation cover and species richness

Plots were afterwards grouped into floristically similar groups using cluster analysis using PAST 2.17c: average linkage agglomeration criterion was applied to a constructed matrix using the

Euclidean distance (Podani 1994). Separate Kruskal-Wallis test were performed to detect possible differences both in diversity, life form and environmental variables on the observed groups.

Both direct and indirect gradient analyses were applied on the dataset using the log transformed species cover data and standardized environmental variables considering also the different group of samples carried out in the cluster analysis. Unconstrained (Correspondence Analysis, CA) and constrained ordination analysis (Canonical Correspondence Analysis, CCA) were employed in order to understand how the variation in the species composition could be explained by ordination (unconstrained) and then to relate species data to the measured environmental variables (constrained ordination). Lepsˇ and ˇSmilauer (2003) suggest to use both unconstrained and constrained ordinations in order to understand how the amount in variability is explained by measured environmental variables.

Ter Braak (1987) recommends to perform previously a detrended correspondence analysis, in order to decide the best model to use in ordination technique between linear and unimodal ones. This analysis helps to assess the length of environmental gradient measured in field.

If the gradient was longer than 4.0 unimodal methods should be used, vice versa a length shorter than 3.0 better fitted with the linear one. Between 3.0 and 4.0 both types of ordination methods work reasonably well. Axes significance was tested with Monte Carlo test (499 permutations). Depending on ordination response, separate regressions were applied on diversity measure with environmental variables and ordination axes. Generalized Linear Models were also built to test the correlation between axes and single species.

4. Diachronic Results

During the 9 temporal recording dates 36 vascular species were sampled (GB1=27; GB2=32; GZ1=23; GZ2=18) (Table 4.1).

Table 4.1 Species recorded during the sampling period with life form and the presence/absence data for each macroplot.

| Species	Life form	GB1	GB2	GZ2	GZ1
Arenaria leptoclados	T scap	0	1	1	1
Asparagus acutifolius	G rhiz	1	0	0	0
Avena sterilis	T scap	1	1	1	1
Brachypodium distachyum	T scap	0	1	1	0
Bromus erecta	H caesp	1	0	0	0
Bromus sterilis	T scap	1	1	1	1
Carduus pycnocephalus	H bienn	1	1	1	1
Catapodium rigidum	T scap	1	1	1	0
Cerastium pumilum	T scap	1	1	1	1
Crepis setosa	T scap	1	0	0	0
Erodium ciconium	T scap	1	1	0	0
Erysimum pseudorhaeticum	H scap	1	1	0	0
Euphorbia helioscopia	T scap	1	1	0	0
Fumaria officinalis	T scap	1	1	1	0
Geranium malacoides	T scap	1	1	1	0
Geranium rotundifolium	T scap	1	1	1	1
Hypochoeris achyrophorus	T scap	0	1	0	1
Medicago minima	T scap	1	1	1	1
Minuartia hybrida	T scap	0	1	1	0
Muscari comosum	G bulb	0	1	0	0
Petrorhagia prolifera	T scap	1	1	0	0
Petrorhagia saxifraga	H caesp	1	1	0	0
Picris hieracioides	H bienn	1	0	1	1
Poa vivipara	H caesp	1	1	1	1
Sanguisorba minor	H scap	1	1	1	0
Saxifraga tridactylites	T scap	1	1	0	1
Sedum album	Ch succ	0	1	1	1
Sedum hispanicum	T scap	1	0	1	1
Sedum rupestre	Ch succ	0	1	0	0
Sedum sexangulare	Ch succ	1	1	1	1
Silene vulgaris	H scap	0	1	1	1
Stachys recta	H scap	1	1	1	0
Teucrium chamaedrys	Ch suffr	1	1	0	0
Tribulus terrestris	T rept	0	0	0	1
Trifolium arvense	T scap	0	0	1	0
Trifolium scabrum	T rept	1	1	0	1
Triticum ovatum	T scap	0	1	0	0
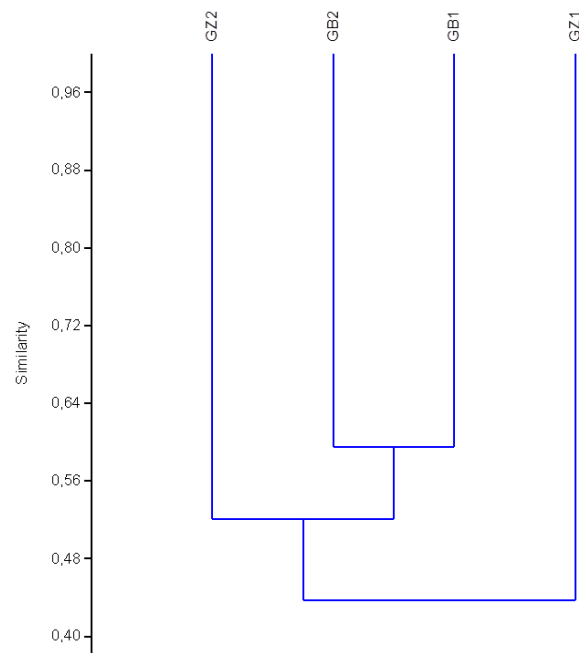
Figure 4.1 Dendrogram of the four macroplots based on the Jaccard similarity index.

GBs macroplots were very similar in species composition, while GZs differed both from GBs and between them (Fig 4.1).The principal dissimilarities were due to differences in *Sedum* species and others such as *Petrhorgia saxifraga, P. prolifera* and *Erysimum pseudorhaeticum*, sampled only in GBs while *Tribulus terrestris, Sedum album* and *Trifolium arvense* were almost exclusively of GZs macroplots (Table 4.1). Concerning *Sedum* species *S. sexangulare* displayed the highest relative cover in GBs plots (respectively 34% and 20% on average) while *S. album* and *S. hispanicum* dominated in GZ2, in particular the first in relative cover (31%) and the second in patches number (averaging 63 over all time). It has to be noted that therophytes (i.e. *Sedum hispanicum, Saxifraga tridactylites*, *Cerastium pumilum, Arenaria leptoclades),* are the most represented life form (about the 60% of the total species) with no significant differences observed among macroplots (Fig 4.2). The vegetation relative cover varied in all temporal stages from 4 to 59% (average 20% ±14% SD) (Table 4.2). The maximum relative cover occupied by a single species in a single macroplot is about of 33% for *Bromus sterilis* on GZ2 (December). On average, however, the maximum values reached by the species were <5% of the entire macroplot extent. GZ2 and GB1 maintained, on average for each month, between the 10% and 21% of more vegetation cover respect to the others sampling sites (p value <0,003). Lower richness values (averaging 4,8 species less) were displayed only in GZ1 trend (p<0,0005) (Fig 4.3).
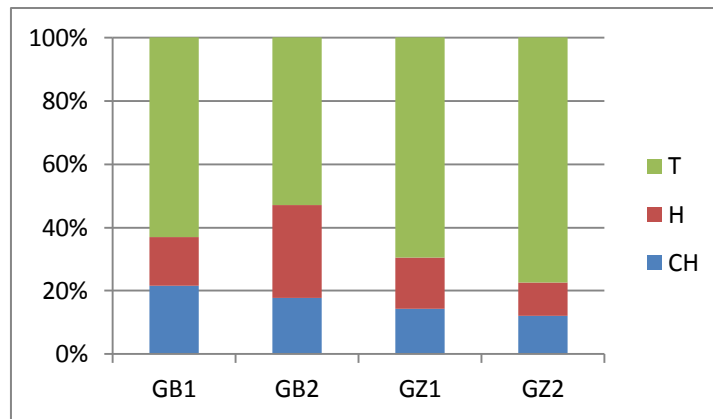
Figure 4.2 Raunkiaer's life form specters of the four macroplots. T= therophyte; CH=chamephyte; H=Hemicriptophyte

Although these differences all of the four macroplots showed similar trends in species, cover, turnover and richness during the sampling period. In all the macroplots richness, cover, and diversity reached the highest values in late winter and spring and a minimum peak during summer (Table 4.2 and figures 4.3).

Table 4. 2 Variables values over the 9 temporal steps. July shows minimum values in all parameters.

	April	May	June	July	October	November	December	February	March
Richness									
GB1	16	18	13	**3**	14	14	16	15	17
GB2	17	12	13	**4**	15	16	16	16	17
GZ1	6	6	7	**3**	9	12	13	13	11
GZ2	13	17	15	**6**	13	14	13	15	16
Shannon diversity									
GB1	0,53	0,45	0,39	**0,16**	0,68	0,87	1,06	1,07	1,21
GB2	0,54	0,39	0,44	**0,17**	0,48	0,55	0,42	0,51	0,79
GZ1	0,24	0,26	0,25	**0,08**	0,39	0,56	0,68	0,82	0,5
GZ2	0,59	0,85	1,06	**0,26**	1,01	1,08	1,09	1,33	0,96
Cover (%)									
GB1	16,6%	12,7%	10,0%	**4,5%**	19,5%	25,8%	33,8%	34,0%	39,3%
GB2	14,4%	10,3%	11,4%	**4,1%**	12,2%	14,2%	9,3%	13,0%	21,2%
GZ1	6,1%	7,4%	6,4%	**2,0%**	11,0%	15,1%	19,4%	23,7%	13,7%
GZ2	17,6%	21,8%	30,6%	**7,5%**	42,7%	48,2%	53,7%	59,3%	45,0%
Number of patches									
GB1	970	799	269	**65**	328	321	421	540	455
GB2	844	171	92	**38**	144	290	355	427	373
GZ1	243	177	146	**113**	284	466	758	903	291
GZ2	251	249	339	**92**	333	549	526	870	438
Turnover									
GB1	52,4%	42,9%	42,1%	76,9%	93,8%	35,3%	12,5%	6,3%	17,6%
GB2	64,0%	47,4%	68,4%	69,2%	68,8%	6,3%	22,2%	11,8%	16,7%
GZ1	58,3%	50,0%	55,6%	57,1%	66,7%	50,0%	7,7%	0,0%	28,6%
GZ2	55,0%	42,1%	31,6%	57,1%	64,3%	20,0%	7,1%	13,3%	17,6%

Decreasing in diversity in summer period was also accompanied by patchiness drop. The maximum number of patches was reached between February and April depending on the macroplots (respectively GZs and GBs) (Fig 4.9). Patch dynamics, however, were often related with single species. Those which display the higher number of patches were *Saxifraga tridactylites, Cerastium pumilum, S. sexangulare* and *S. hispanicum,* while *Bromus sterilis, Stachys recta*, *Carduus pycnocephalus* and *Avena sterilis* display the highest dimension per patch.
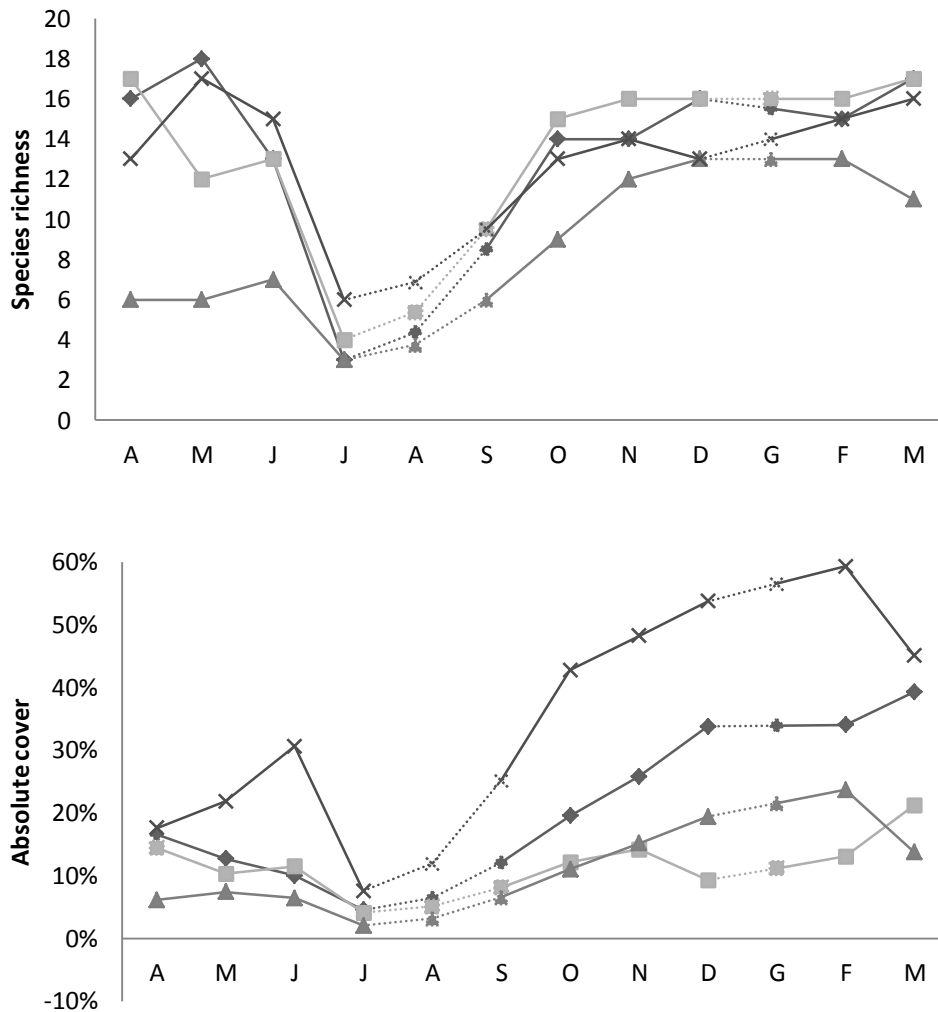
Figure 4.3 Inter annual trends of the considered variables in the four macroplots. Dotted lines represent the estimation of trend during the lacking sampling periods. □=GB1; ■=GB2; ▲=GZ1; □=GZ2 (continue)
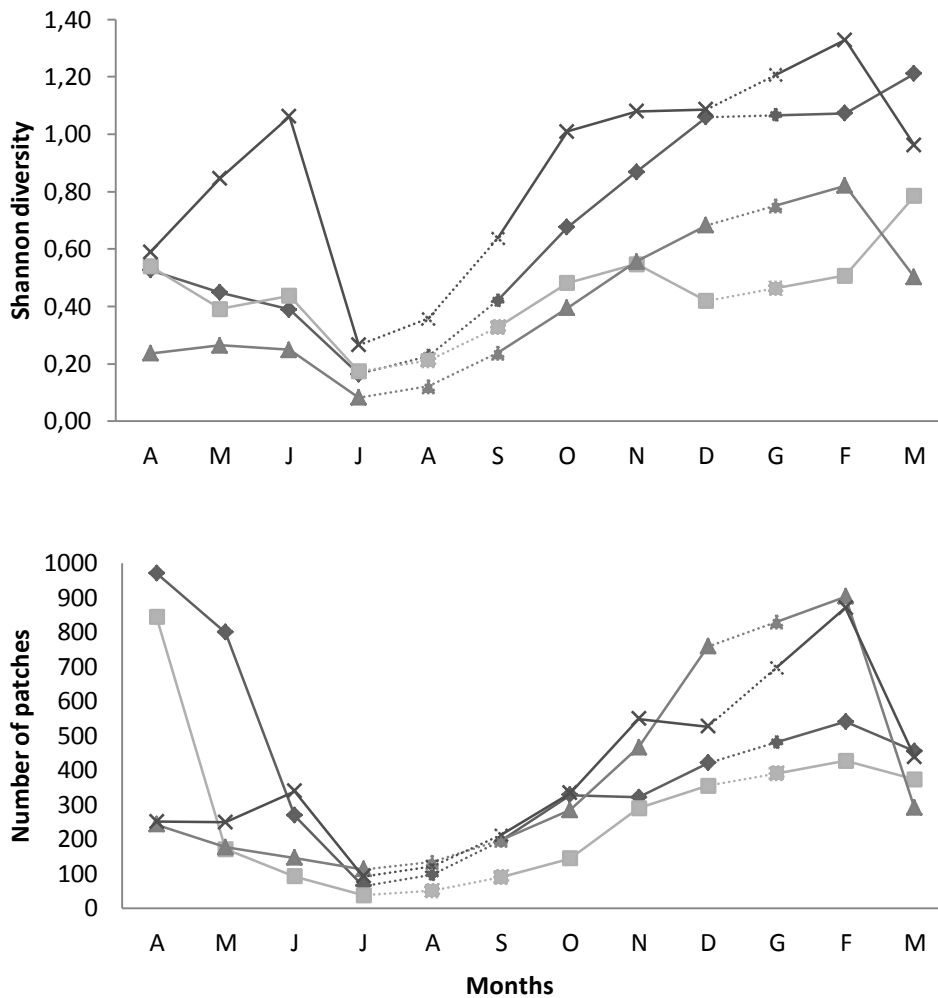
Figure 4.4 Inter annual trends of the considered variables in the four macroplots. Dotted lines represent the estimation of trend during the lacking sampling periods. ◆=GB1; ■=GB2; ▲=GZ1; ✕=GZ2

These trends were accompanied by high species turnover, similar for the 4 macroplots, both between pairs of steps and considering the whole period. Species pools of April 2012 and March 2013 differed about of 57%. Spring months (Fig. 4.4) and the early autumn's ones exhibited major changes in species composition (from 60% to 90% of the whole pool), due principally to the drop of diversity in summer and its following recover. Winter represented the most stable period with only 15% of turnover; however also May and June displayed high diversity and simultaneously relative low value in turnover index ($May_{TO}=46\%$ and $June_{TO}=30\%$).
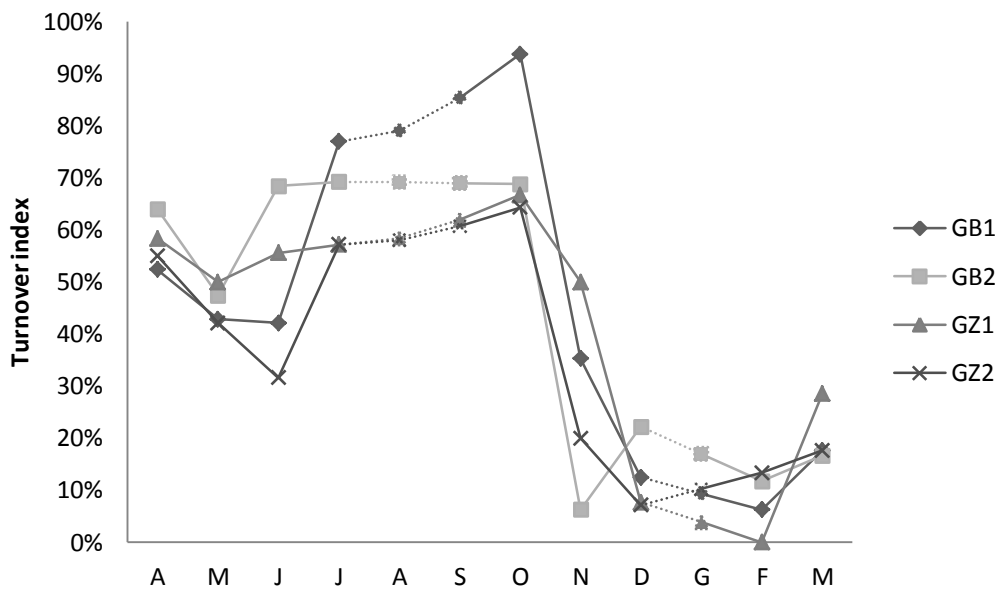
Figure 4. 5 inter annual trends of the turnover in species in the four macroplots.

In this process a key role were played by therophyte's temporal dynamics (Fig 4.5). These, indeed, in term of patches number and cover, increased immediately after dry period, while chamephytes and hemicryptophytes were less affected by this seasonal event.
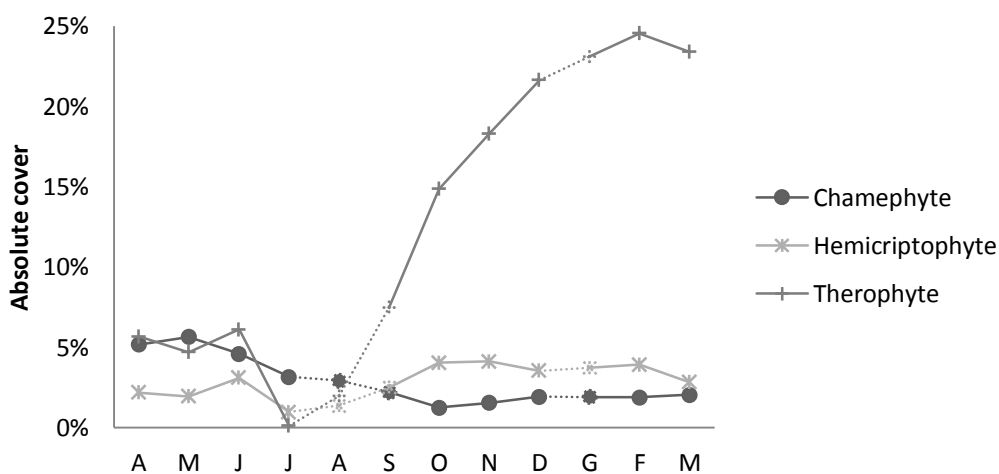

Figure 4.6 Inter annual trend of different life form.

The regressions between turnover index, diversity values and temperatures showed a clear dependence of the two ecological variables with this meteorological factor (table 4.3). Change in species pool (both in appearances and disappearances) increased with temperatures (fig.4.6) while an inverse relationship was shown by diversity (Fig 4.7) and richness. By contrast, mean precipitation values show a weak significant relationship only with diversity and richness, however the portion of explained variance was extremely low (Table 4.3).

Table 4.3 Linear regressions of variables with climatic parameters

	Mean temepratures			Precipitations		
	Estimate	R^2	P|t|	Estimate	R^2	P|t|
Richness	-0,36	0,34	<0,0005	1,29	0,19	0,01
Shannon index	-0,02	0,55	<0,0005	0,08	0,11	0,05
Turnover	0,02	0,54	<0,0005	-0,05	0,09	0,08

a.   b.
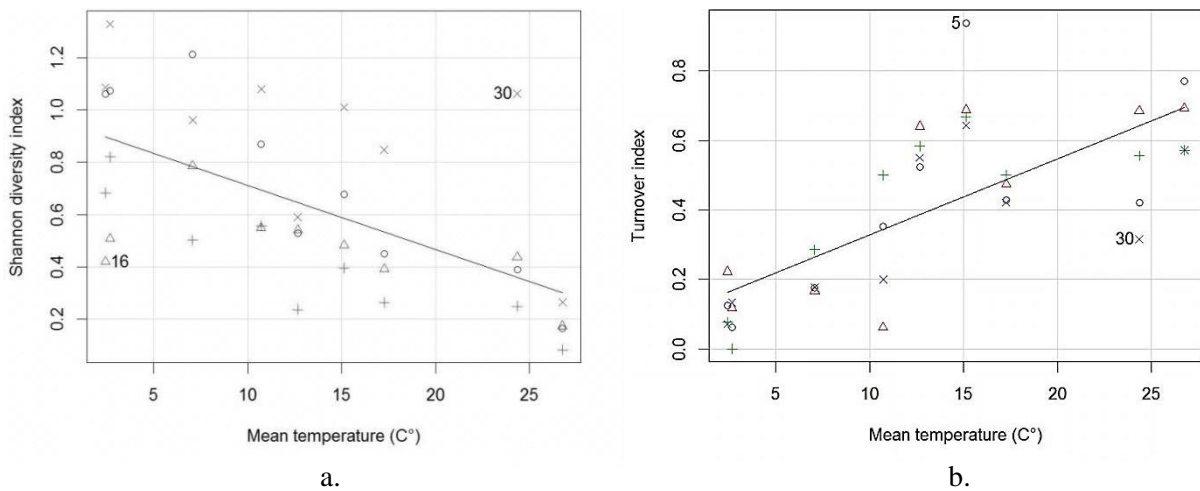
Figure 4.7 Correlation between turnover (a) and Shannon indices (b) with mean temperature. ○=GB1; △=GB2; +=GZ1; ✕=GZ2.
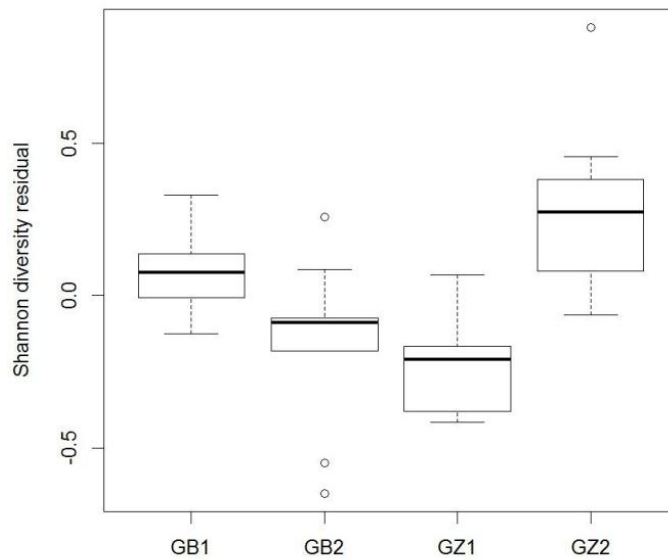
Figure 4. 8 Boxplot of residuals of the regression between Shannon index and mean temperatures. GZ2 macroplot showed higher values. (F=9,0,2, p<0,0001)

The ANOVA based on the residual values of diversity of the regression with temperatures between macroplots confirmed the differences in maintaining mean diversity value during the year (Fig. 4.7).

CA analysis on log transformed principal species cover and months were in Figure 4.8. The first two axes of the ordination explained almost the 80% of variance (68% the first axis, and 11% the second axis). The first one clearly differentiated the autumn-winter seasons from the dry seasons. The biplot shows an early spring species cluster composed by the therophytes *Cerastium pumilum,* and *Saxifraga tridactylites,* a late spring one formed by *Sedum album, Petrorhagia saxifraga* and *S. sexangulare* and a group with autumn and winter species such as *Bromus sterilis, Arenaria leptoclados, Carduus pycnocephalus* and *Poa vivipara.*
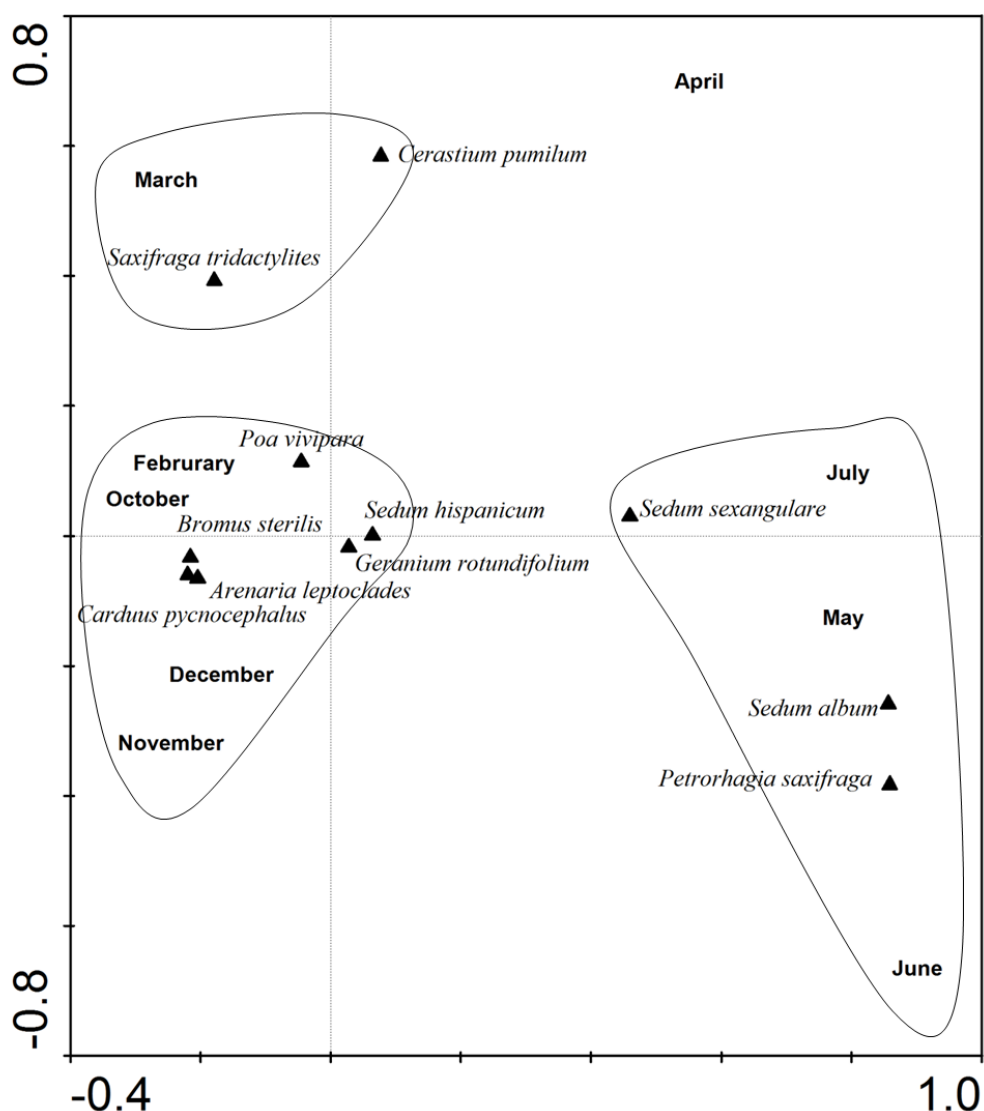

Figure 4. 8 Biplot of the CA showing the clear separation between warm months and the coldest ones. Triangles display the maximum cover of the species. Eigenvalues: Axis 1= 0,55; axis 2= 0,10.
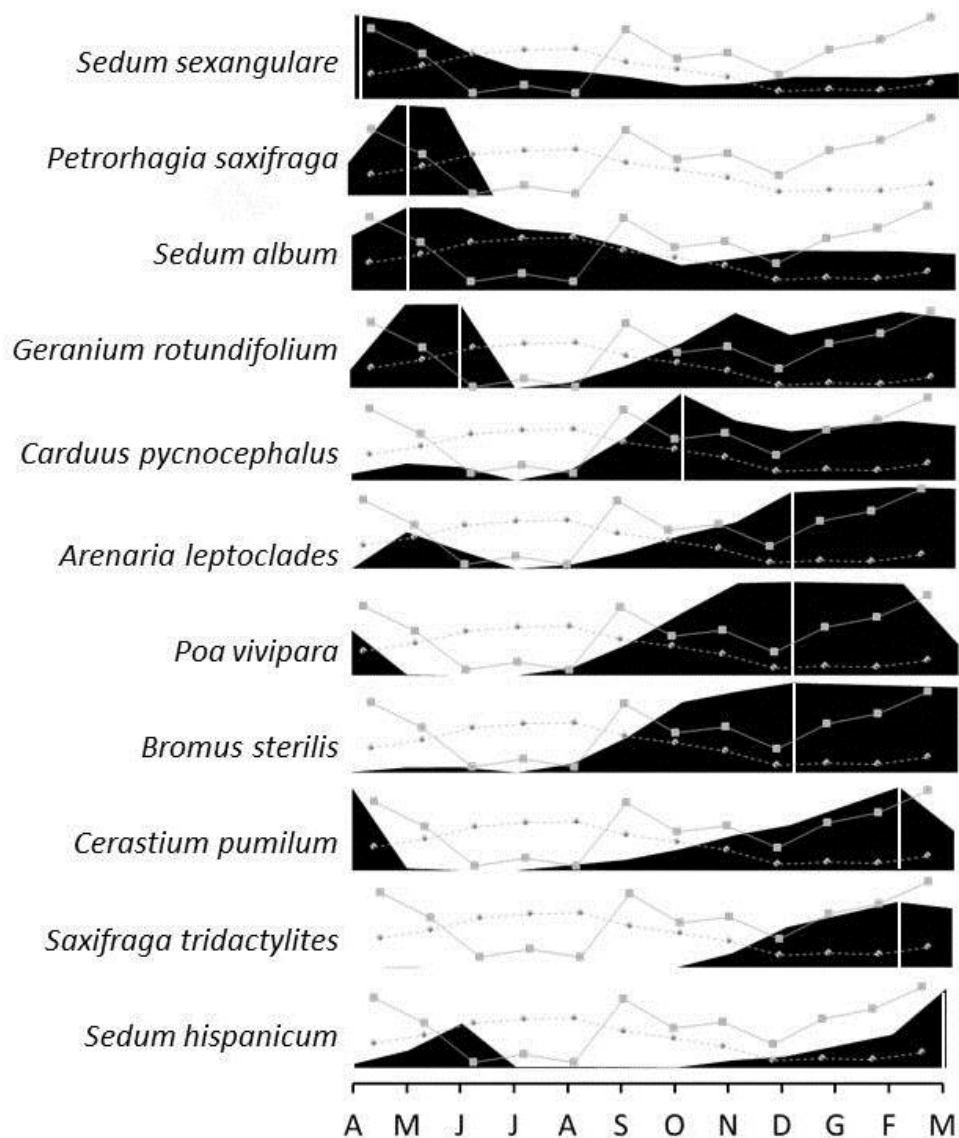
Figure 4.9 Cover trend over the whole sampling year of principal species with climatic trends. Dotted line indicates temperature; continue line indicates precipitation. The white lines show where the species reach the maximum cover.

The inter annual trend of the principal species it well showed by Figure 4.9 that indicated the cover trend of the principal species on the whole period joined with mean temperatures and rainfall trends to highlight different pattern in species seasonal occurrences.

Figure 4.10 shows the sequence of the vegetation cover in the subplot frequency layers of GB1 macroplot over the sampling period used in the construction of the GLMM.

Postulating time-varying coefficients implies that effect of substrate typology may change over time: this was a crucial assumption to be checked in this case study that aims at evaluating the

evolution (if any) along time of the suitability of different substrates. Random effects α_t, θ_s and δ_{st} are respectively devoted to capture temporal, spatial and spatiotemporal variability.
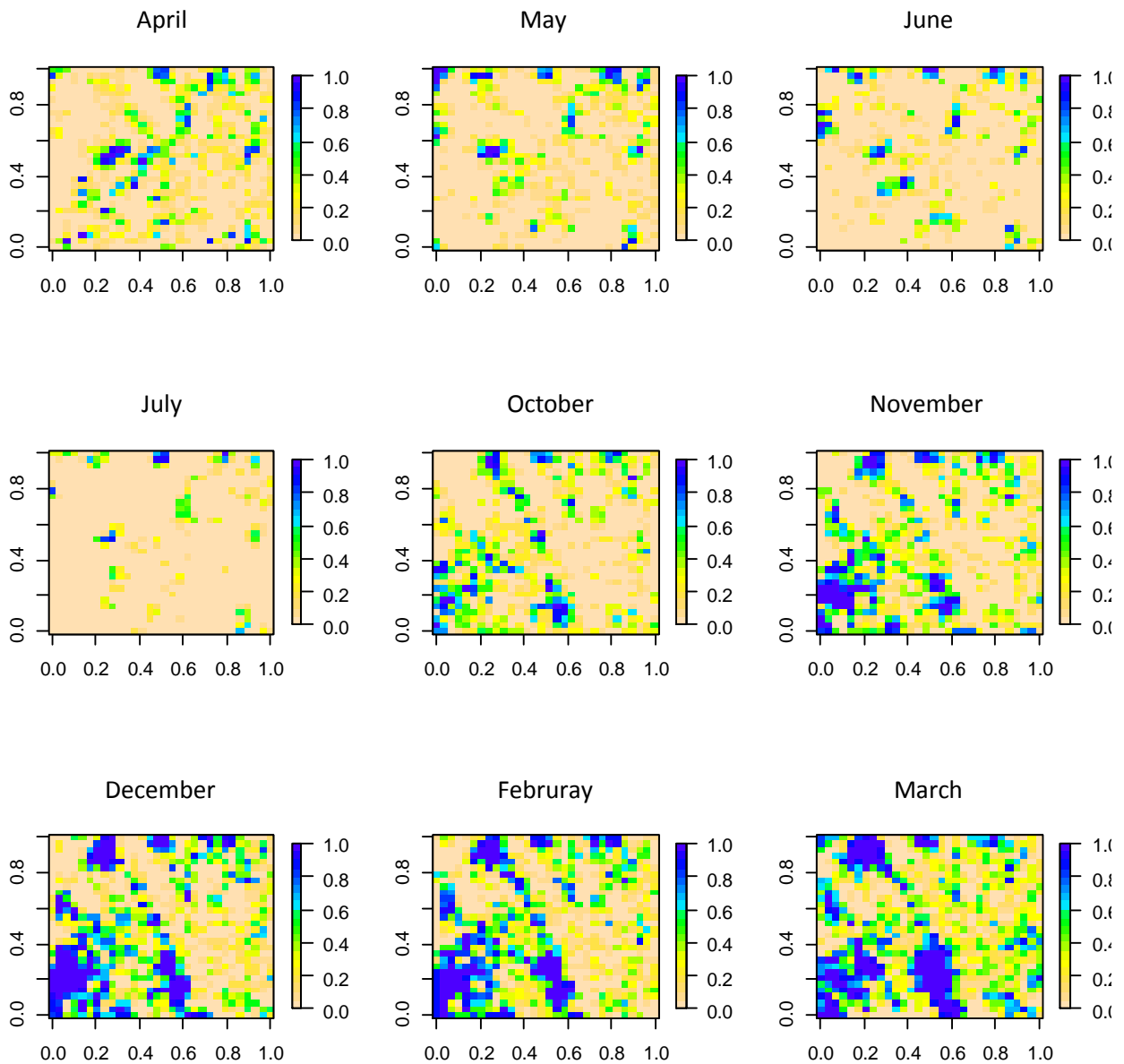

Figure 4.10. Subplot frequency layer of vegetation cover in GB1 macroplot over the 9 temporal steps.

Figure 4.11 shows the effect of each substrate on vegetation cover. It is important to note that the effects should be considered jointly, i.e. they should be interpreted as measures of relative suitability. A positive coefficient implied that the substrate was more suitable with respect to the average suitability. Hence, bryophyte reveals as the most suitable substrate at the beginning of the study period and its relative suitability is almost constant along time. On the other hand, soil increases its relative suitability along time and at the end of the study period its relative suitability is significantly higher than the average. On the contrary, bare rock it's the less suitable and shows a

decreasing pattern. As an opposite behavior significantly higher than zero and constant across time, meaning that moss is a suitable type of substrate for the plant and has the same sort of effect during the whole period. Finally, litter shows a constantly average suitability along time.
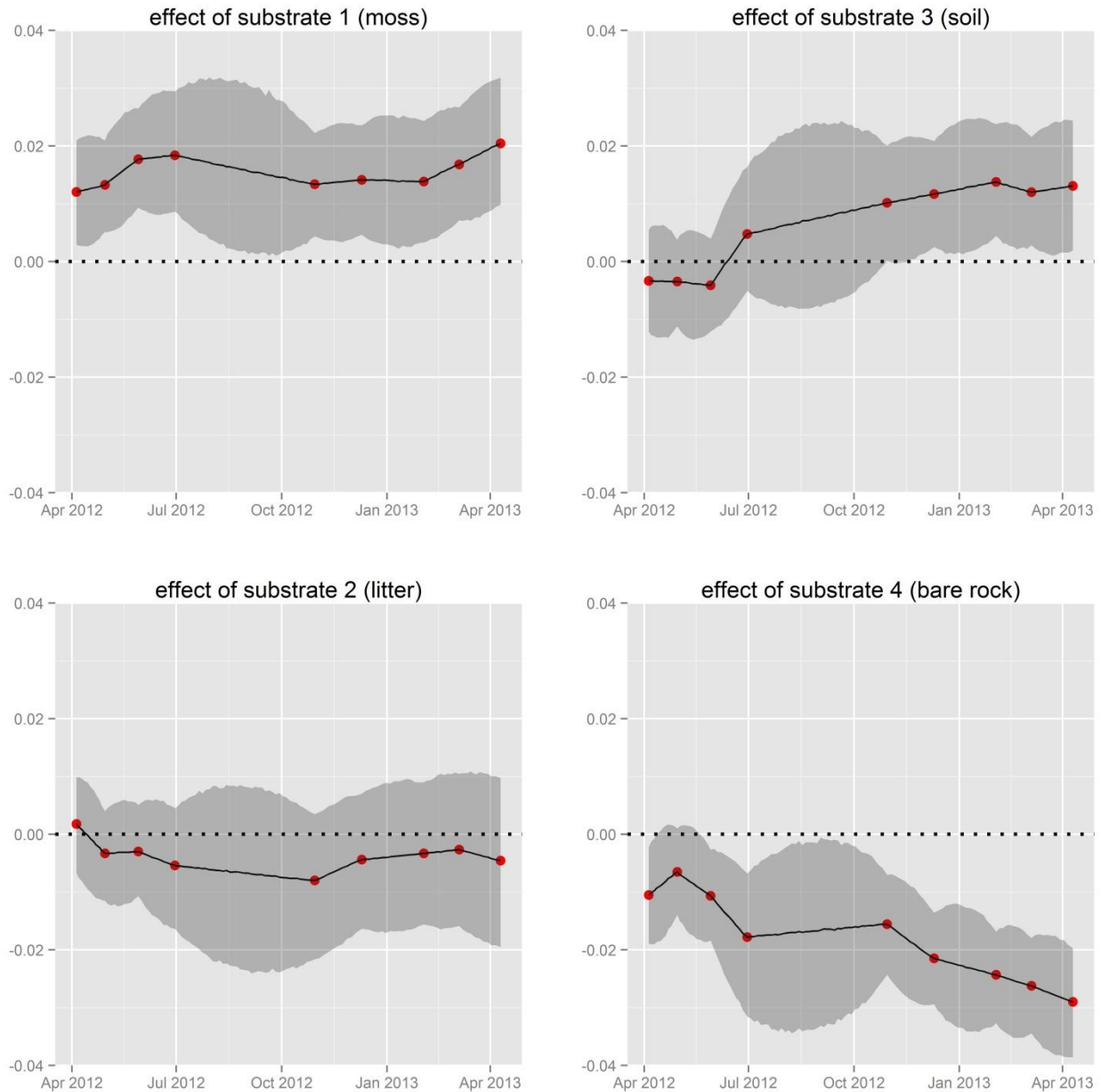

Figure 4.11. The substrate effect on vegetation plotted against time. For each substrate, the effect estimated at observed times (black dots) and predicted at unobserved regularly spaced times (red lines). 90% confidence bands in grey.

5. Synchronic Results

A total of 46 species were recorded during the survey. Sample rarefaction curve (Fig 5.1) showed that the 98% of all of the species were sampled at the 88th plot, however the curve didn't reach the asymptote at the 99th plot. Most represented life form was therophyte (46%), followed hemicryptophytes (28%) and chamaephyte (26%).
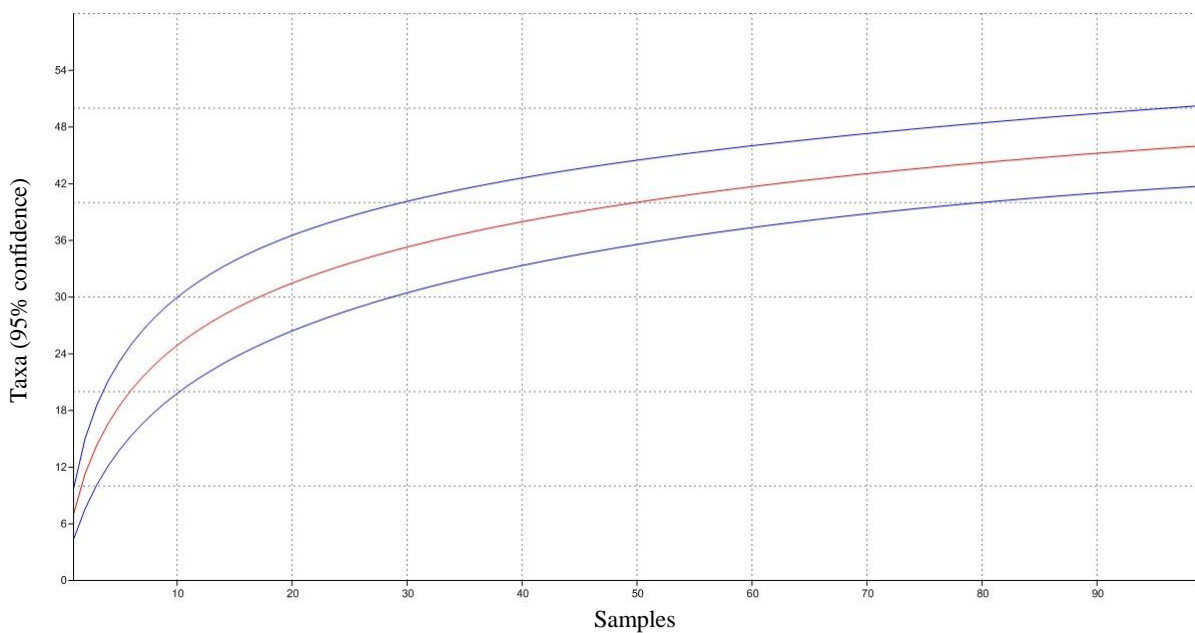

Figure 5. 1 Sample rarefaction curve shows how many new taxa were found for each plot sampled

Species richness per plot ranged between 1 and 14 (average 7,2 ± 3). Most of the species showed low frequency values, according with other studies in rocky garigues (see Chiarucci 2001 for example). Only 4 species (8% of the total) were found in more than 50% of the plots, 20 (43%) were recorded in five or less plots. *Sedum hispanicum* was the most frequent species (75 plots) followed by *Arenaria leptoclados, Cerastium pumilum, Saxifraga tridactylites* and *Sedum album* (from 67 to 42 plots) (Table 5.1). Vegetation cover varied between 6% and 63% (average 30% ±14). The species that reached the highest values in absolute cover were *Sedum hispanicum* (maximum cover of 50%), *S. album* and *S. sexangulare* (about 35%). The half of the species, however, didn't reach the 5% of cover in any plot (Table 5.1).
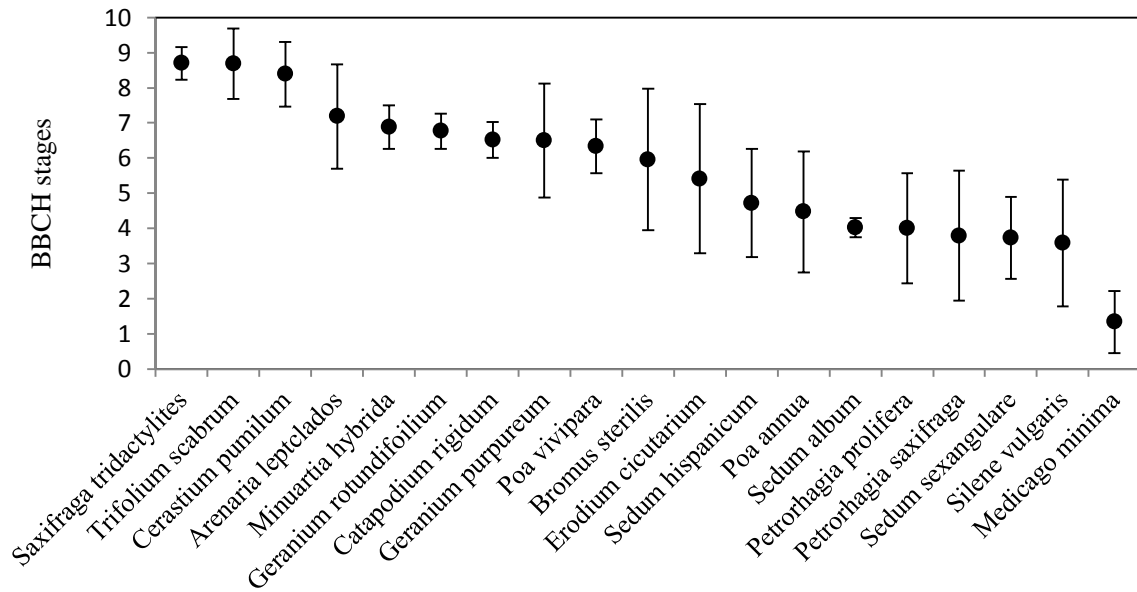
Figure 5. 2 Mean phenological stages of each species according with BBCH scale.

According with diachronic analysis (Fig 4.8), species showed differences also in phenological stages. *Saxifraga trydactilites, Trifolium scabrum* and *Arenaria leptoclades* exhibited advanced phonological stages, near to senescence (8-9 in BBCH stages), while *Silene vulgaris, S.sexangulare* and *S.album* present them in full vegetative period (stages 3-4) (Fig. 5.2).

Rock cover was significantly positively correlated with slope (R^2=0.24; p<0,0005) as well as convex plots had, on average, less soil respects the other topographical features (Kruskal Wallis χ^2=10.5; p<0,01) (Fig 5.3) .

Table 5. 1 Species recorded during synchronic sampling. Frequency, maximum cover reached in a single plot and mean cover on 99 plots are shown. Black line shows the threshold for the species considered in further analysis.

|	Life form	Frequency (%)	Maximum cover (%)
Sedum hispanicum	T scap	75	50
Arenaria leptclados	T scap	66	15
Cerastium pumilum	T caesp	65	20
Saxifraga tridactylites	T scap	63	25
Bromus sterilis	T scap	45	20
Sedum album	Ch suff	42	36
Geranium rotundifoilium	T scap	34	7
Poa vivipara	H caesp	30	5
Trifolium scabrum	T rept	25	8
Catapodium rigidum	T scap	23	4
Sedum sexangulare	Ch suff	22	35
Silene vulgaris	H scap	19	7
Erodium cicutarium	T scap	17	10
Minuartia hybrida	T scap	16	3
Poa annua	T caesp	15	4
Pethroragia prolifera	T scap	14	10
Pethroragia saxifraga	H caesp	14	10
Medicago minima	T scap	12	2
Geranium purpureum	T scap	12	15
Hieracium	H caesp	8	3
Triticum ovatum	T scap	8	25
Trifolium arvensis	T scap	8	5
Carduus pycnocephalus	H Bienn	6	1
Sanguisorba minor	H scap	6	3
Geranium malacoides	T scap	6	8
Lotus corniculatus	H scap	6	15
Erysimum pseudorhaeticum	H scap	5	5
Crepis setosa	T scap	4	1
Stachys recta	H caesp	4	1
Sedum rupestre	Ch suff	3	27
Euphorbia exigua	T scap	3	1
Avena sterilis	T scap	3	1
Euphorbia helyscopia	T scap	2	1
Geranium dissectum	T scap	2	1
Galium mollugo	H caesp	2	1
Vulpia ciliata	T caesp	2	6
Bombycilaena erecta	T caesp	2	5
Ajuga chamaepitys	T scap	2	1
Bromus erectus	H caesp	1	1
Galium lucidum	H scap	1	1
Galium aparine	T scap	1	1
Linum strictum	T caesp	1	1
Helichrysum italicum	Ch suff	1	1
Muscari comosum	G bulb	1	1
Salvia pratensis	H scap	1	1
Thymus longicaulis	Ch rept	1	2

a.

b.
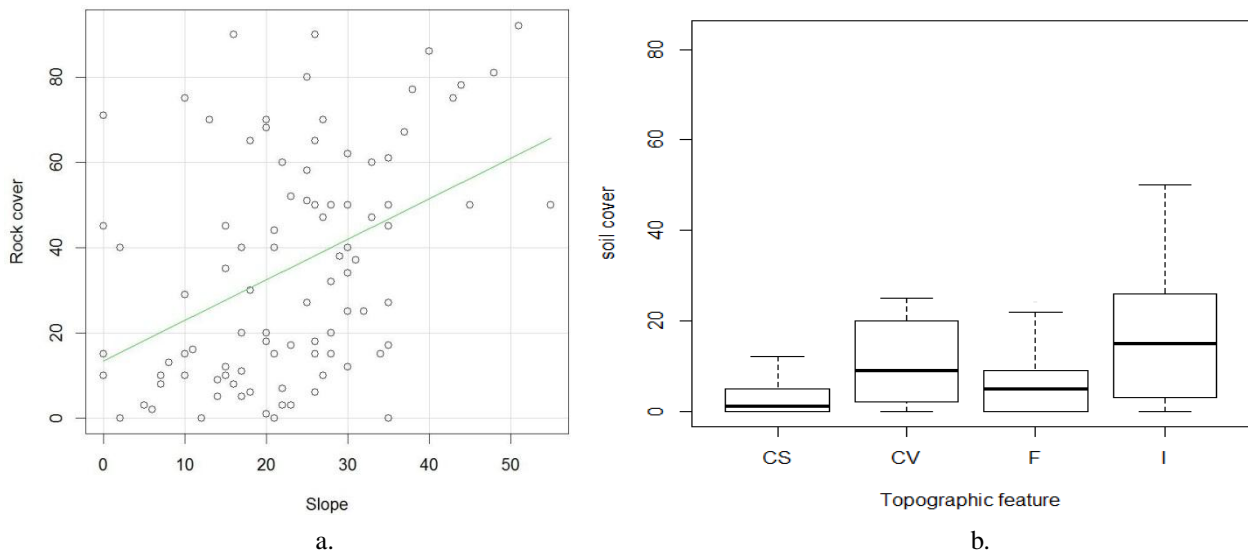
Figure 5.3 Relationships between environmental variables. In (a) the regression of rock cover on slope; in (b) the boxplot displays the effect of the topographic feature on the soil cover. CS= convex; CV=concave; F= flat; I=irregular.

Table 5.2 Results of the Kruskal-Wallis test of the diversity variables on environmental factors

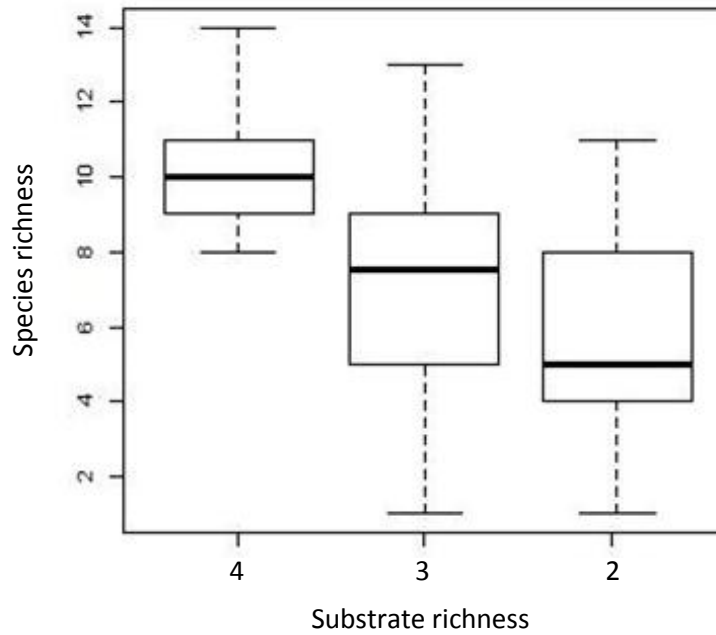| Variables	Envionmental factors	$\chi 2$	P<0,05
Richness	Substrate richness	11,97	**<0,0001**
	Topograpfhic features	21,99	**<0,0001**
	Tramplig	3,23	0,07
Vegetation cover	Substrate richness	0,311	0,73
	Topograpfhic features	0,68	0,30
	Trampling	4,42	**0,04**
Shannon diversity	Substrate richness	10,44	**<0,0001**
	Topographic features	10,75	**0,01**
	Trampling	0,015	0,90

Figure 5. 4 Boxplot showing differences in species richness between plot with different numebr of substrates.

The analysis of variance showed that substrate richness affected both richness and diversity with marked differences between low and high values as well as topographic features (Table 5.2 and Fig 5.4).

Respect to the other environmental variables, richness was positively correlated only with soil depth (R^2=0,10;p<0,003), while had a linear negative relationship with rockiness (R^2=0,11;p<0,0007) as well as Shannon diversity (R^2=0,28; p<0,0008) (Fig 5.5). Some effect on vegetation cover was due only to human trampling by reducing about of 10% (Fig 5.6).
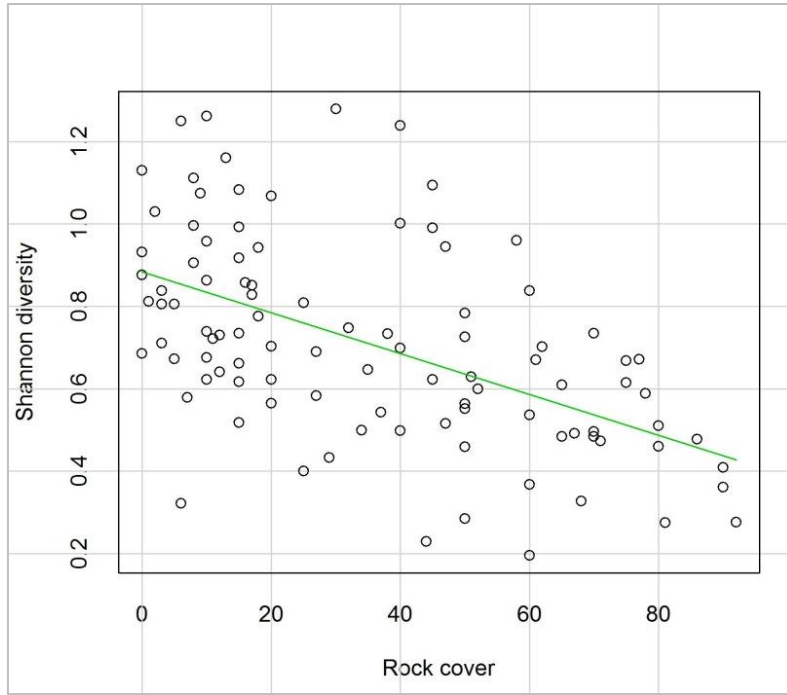
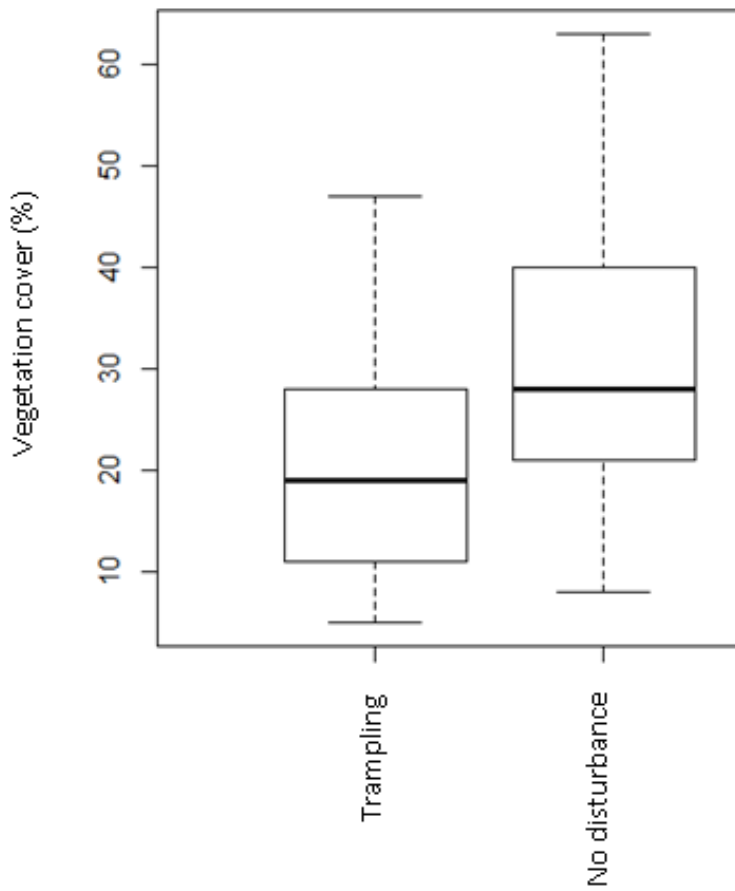**Figure 5.5 Regression between rock cover and Shannon diversity index.**

Figure 5. 6 Effect of human trampling on vegetation cover

However, if species richness were considered in relationships with different life form cover, it responded in different way respect to substrate richness (Table 5.3): in most heterogeneous plots richness increase only with the increasing of theorphytes cover while with only 1 or 2 types of substrate hemocryptophytes leaded species richness.

Table 5. 3 Multiple regressions between life form and richness within substrates richness.

Life-form	Substrate richness	Estimate	t	Pr(>|t|)
Therophyte	4	0,13	4,10	**0,0001**
	3	-0,06	-2,77	**0,0069**
	1-2	0,02	0,67	0,5021
Hemicryptophyte	4	0,14	1,38	0,1707
	3	1,11	3,61	**0,0005**
	1-2	0,37	4,11	**0,0001**
Chamaephyte	4	0,03	0,21	0,8380
	3	0,04	0,63	0,5320
	1-2	-0,01	-0,34	0,7342

Cluster dendrogram shown in figure 5.7 displays two main groups of plots corresponding to the two geographical areas (GZ and GB) with few exceptions. The former group (GR1) was characterized by the presence of *S.album*, and the latter (GR2) by *Petrorhagia saxifraga*. GR1 was also characterized by a higher frequencies in xeric annuals such *Arenaria leptoclados* and *Minuartia hybrida* while GR2 by grassland species such *Medicago minima, Hieracium sp, Sanguisorba minor* and *Erodium cicutarium.* However, the two groups maintained in common some of the most important species for the Habitat 6110* such as *Saxifraga tridactylites, Sedum hispanicum* and *Cerastium pumilum.* Further, it was possible to subdivide GR1 into other two sublcluster due to differences in frequencies of two important species: *S. hispanicum* and *S. album*.

The plots characterized by the presence of *S.hispanicum* ($GR1_{Sh}$) had, on average, lower species richness and diversity values respect to the other (fig 5.8). Plots belonging to GR2 were affected by a higher environmental heterogeneity (Fig. 5.8) while almost all the chamephytes were clumped in the cluster dominated by *S.album*.
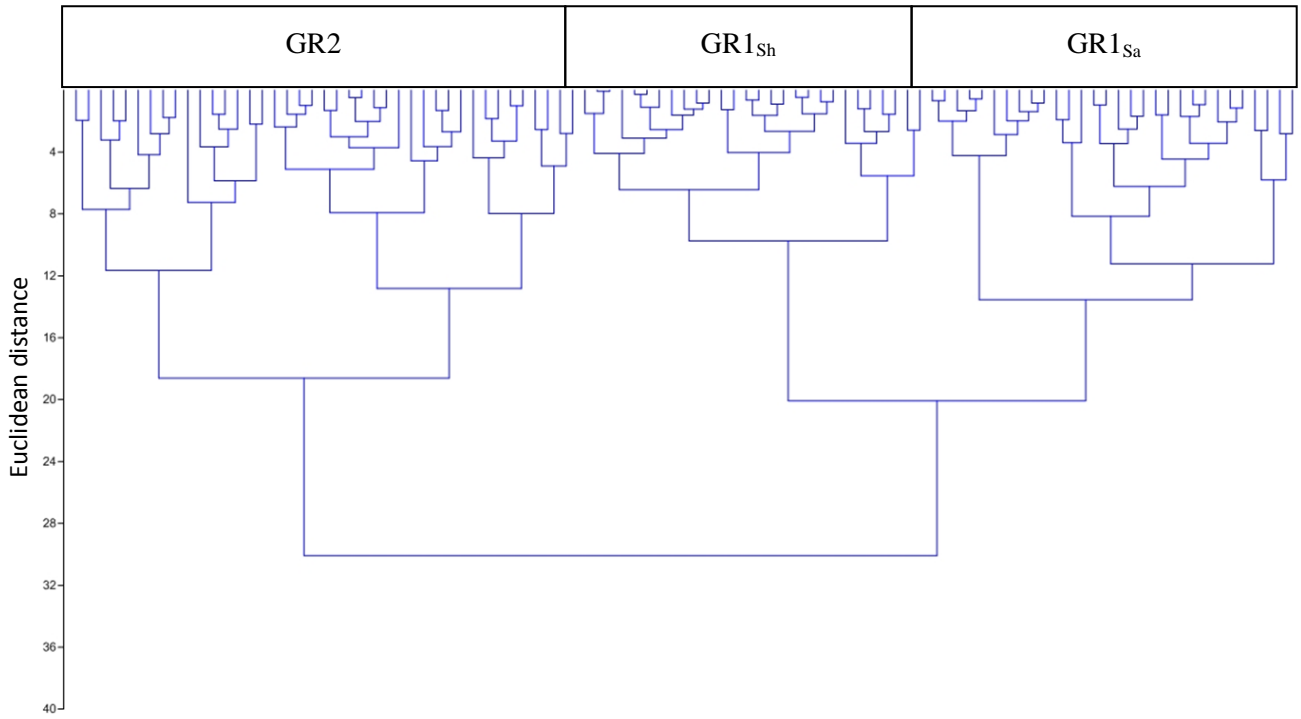
Figure 5. 7 Cluster dendrogram showing the principal groups based on the Euclidean distance of species abundance
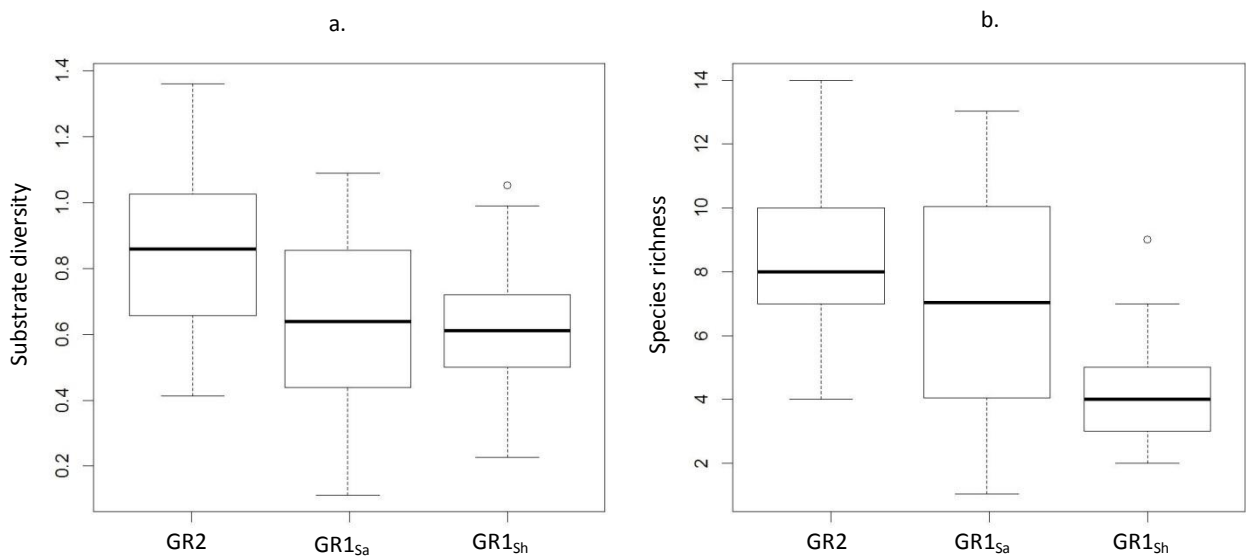

Figure 5. 8 Boxplot displaying the difference in mean values of substrate diversity (a) and species richness (b) within clusters. Kruskal-Wallis values were: richness $\chi 2$= 17,6, p<0,0001; vegetation diversity $\chi 2$=13,4, p<0,001; vegetation cover $\chi 2$=6,4, p<0,03; substrate diversity $\chi 2$=6,15, p<0,0005

Figure 5.9 and Table 5.4 show the CA results. The first two axes described about the 20% of all the variance of dataset. CA confirmed results derived by the cluster analysis with a good separation between groups although the transition from one to another was gradual. GR1$_{Sa}$ and GR1$_{Sh}$ clouds of plots were associated with few other species, such as *Arenaria leptoclados, Minuarthya hybrida, Poa vivipara* and *Trifolium arvese*. The species with higher scores on the first axe, such as *Erodium cicutarium, Medicago minima, Geranium malacoides., Erysimum pseudorhaeticum* and *Sanguisorba minor*, had a wider ecological range and, however, were more afferent to semi-arid grassland.
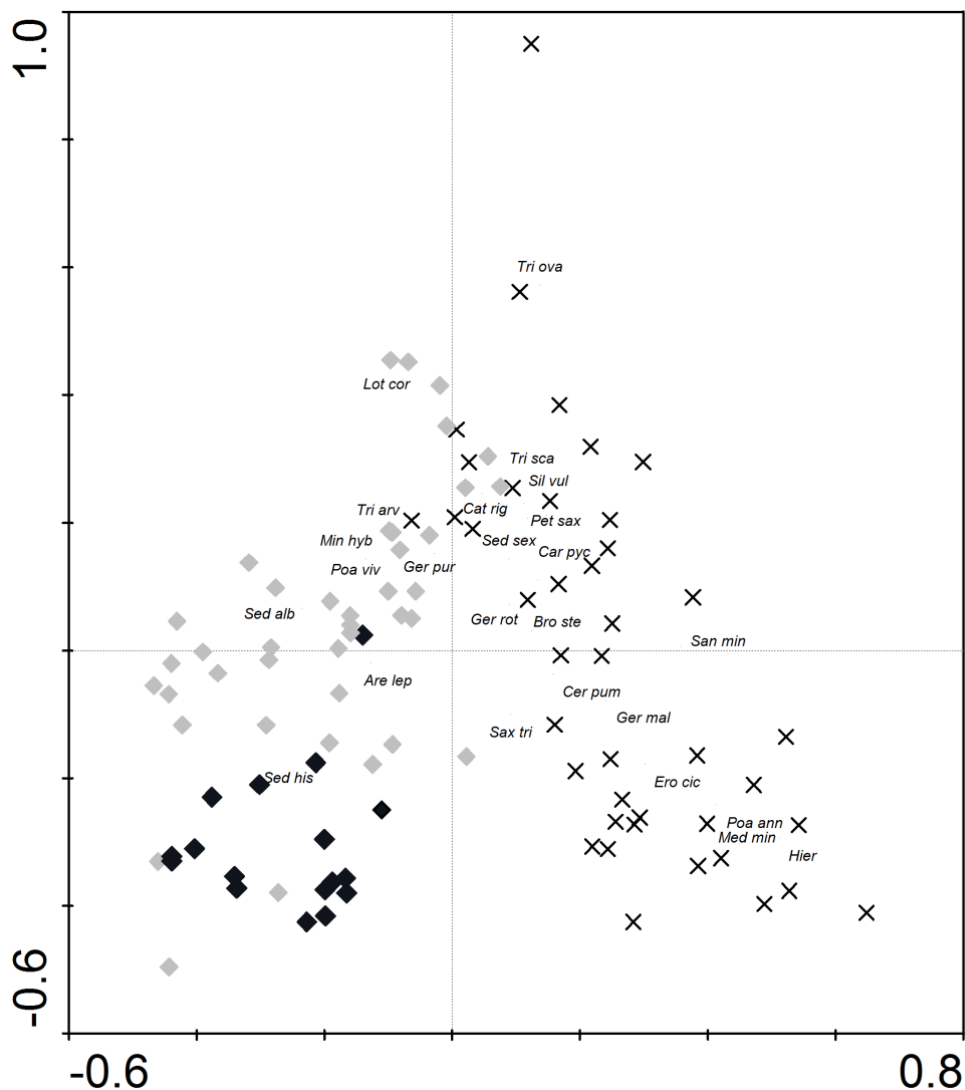

Figure 5. 9 CA biplot of species and sample organized in clusters. ◆=GR1$_{Sh;}$ ◆=GR1$_{Sa;}$ ✕=GR2. Eigenvalues: first axis =0,47, and second axis = 0,28

Table 5.4 Summarize table of CCA

Axes	1	2
Eigenvalues	0.314	0.110
Species-environment correlations	0.879	0.628
Cumulative percentage variance		
of species data	11.8	16.0
of species-environment relation	48.3	65.3

CCA biplot of species and samples with environmental variable are shown in figure 5.10. The Monte Carlo permutation indicated that all the canonical axes were significant (p value < 0.001). The first two axes of CCA explain about a 16% of the variance of the species with a good correlation with the environmental variables (87% for the first axe and 62% for the second) (tab 5.4).

The biplot showed some differences in plots organization with those of the CA as well as eigenvalues were lower too. This could be due to the lack of some variables not considered in the study. The first axis indicated an increase in slope and the rockiness and a decrease in soil depth and cover and north-west faced exposure while the second one describes an inverse relationship between shadow and bryophyte mat (Table 5.5).

Table 5.5 Inter set correlations of environmental variables with axes

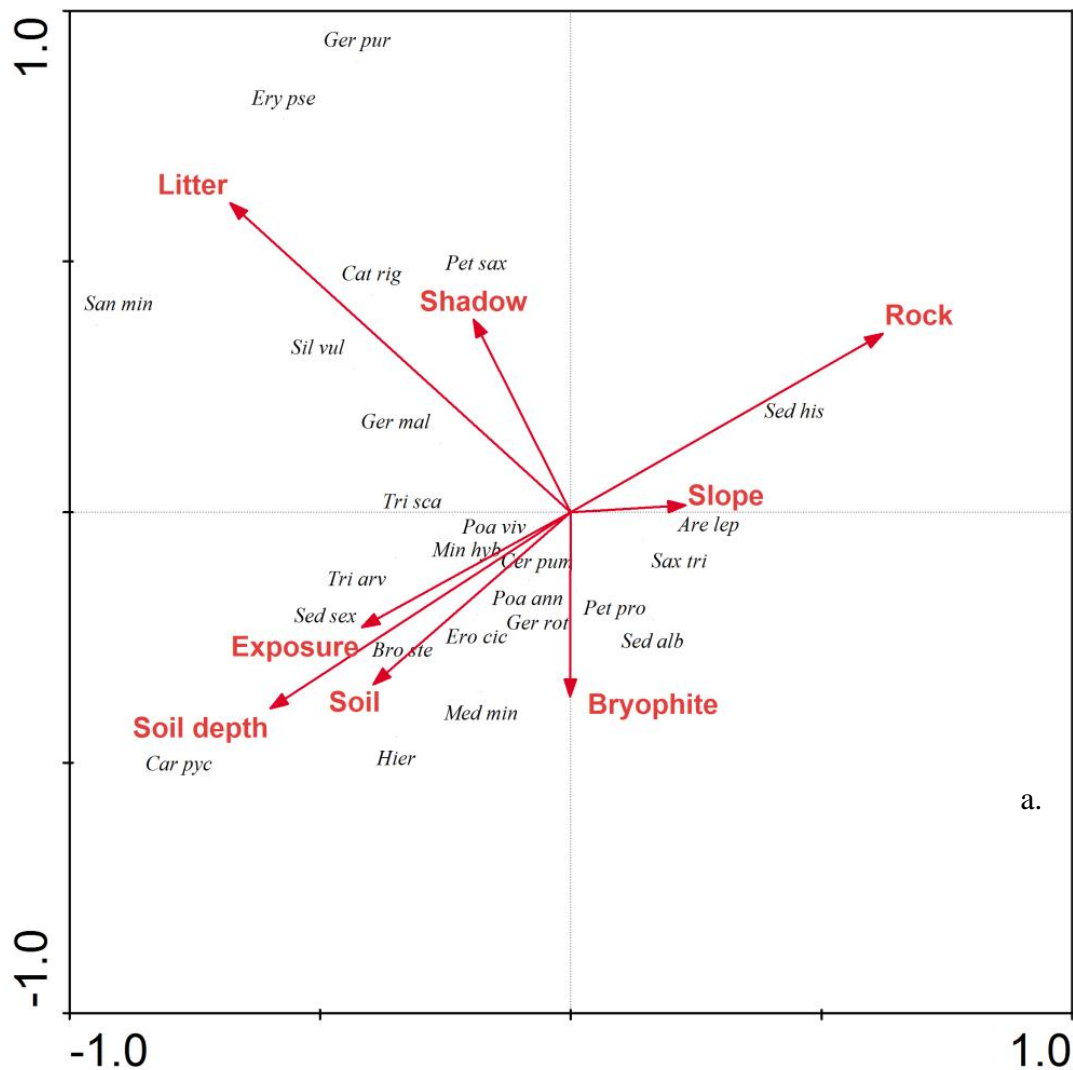| Variables	AX1	AX2
Bryophyte cover	0,005	-0,41
Rock cover	0,50	0,21
Litter cover	-0,42	0,52
Soil cover	-0,29	-0,21
Exposure	-0,29	-0,12
Mean soil depth	-0,37	-0,20
Slope	0,18	0,001
Shadow	-0,13	0,28

Figure 5. 10(a) Biplot of CCA of species with standardized environmental variables. Soil, litter, bryophyte and rock are to intend as cover. Species legend: (*Are lep*) *Arenaria leptoclados;* (*Bro ste*) *Bromus sterilis;* (*Car pyc*) *Carduus pycnocephalus;* (*Cat rig*) *Catapodium rigidum;* (*Cer pum*) *Cerastium pumilum;* (*Ero cic*) *Erodium cicutarium;* (*Ery pse*) *Erysimum pseudorhaeticum;* (*Ger mal*) *Geranium malacoides;* (*Ger pur*) *Geranium purpureum;* (*Ger rot*) *Geranium rotundifolium;* (*Hier*)Hieracium spp.; (*Med min*) *Medicago minima;* (*Poa ann*) *Poa annua;* (*Poa viv*) *Poa vivipara;* (*Pet pro*) *Petrorhagia prolifera;* (*Pet sax*) *Petrorhagia saxifraga;* (*Sax tri*) *Saxifraga tridactylites;* (*Sed alb*) *Sedum album;* (*Sed his*) *Sedum hispanicum;* (*Sed sex*) *Sedum sexangulare;* (*San min*) *Sanguisorba minor;* (*Sil vul*) *Silene vulgaris;* (*Tri arv*) *Trifolium arvense;* (*Tri sca*) Trifolium scabrum.
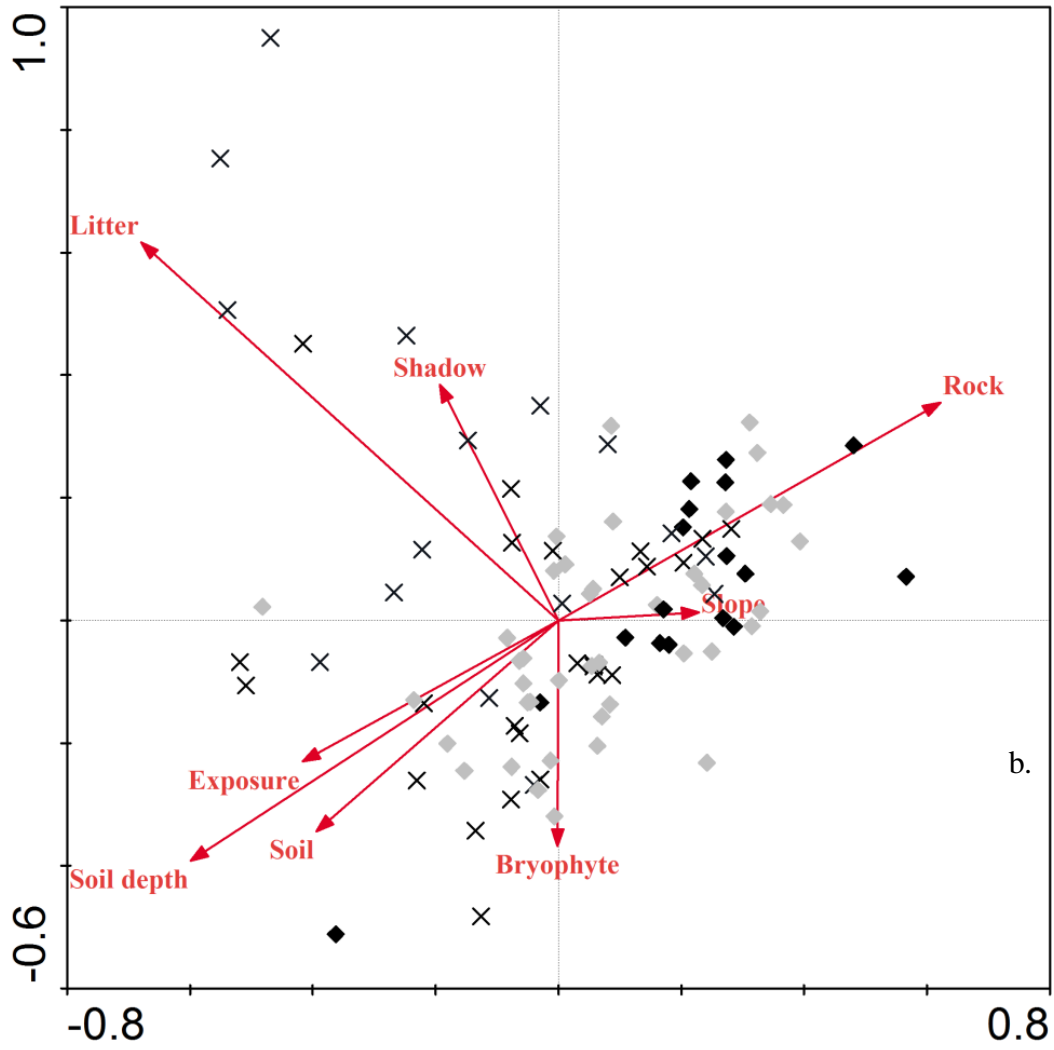
Figure 5. 11(b) Biplot of CCA with standardized environmental variables. Soil, litter, bryophyte and rock are to intend as cover.. ◆=GR1$_{Sh}$; ◆=GR1$_{Sa}$; ✕=GR2.
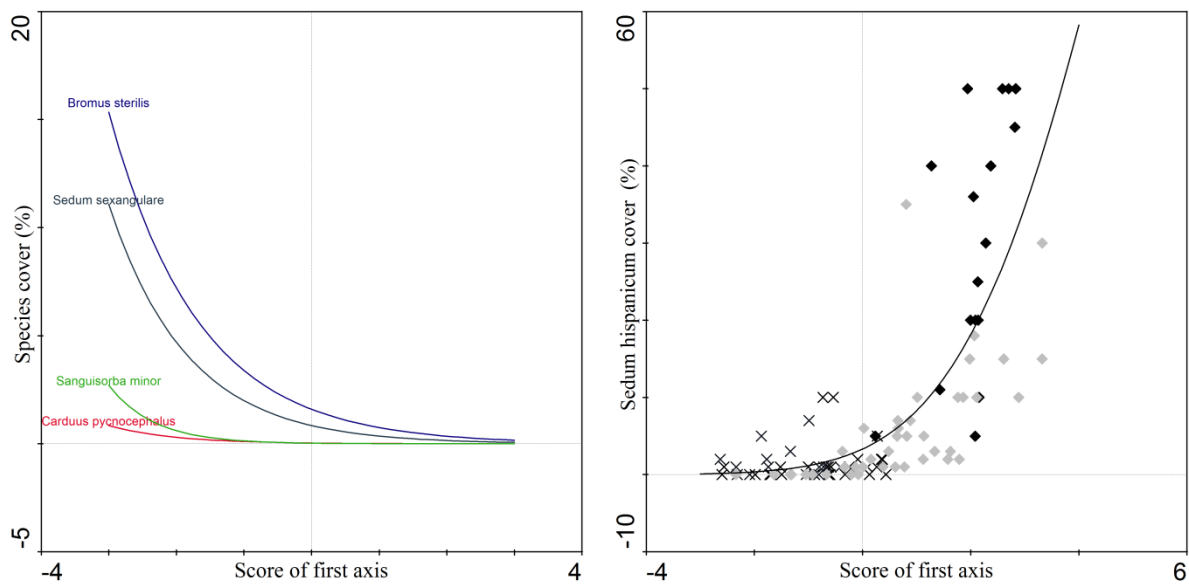
Figure 5. 12 Species cover response curve on the first axis

GR1$_{Sh}$ plots were well grouped on the right with *S.hispanicum* which displayed a strong positive relationship along the first "rocky" axis. Otherwise species like *Sanguisorba minor, Bromus sterilis, Sedum sexangulare* and *Carduus pycnocephalus* were associated with more developed soil stages (Fig. 5.11) (Table 5.6). GR1$_{Sa}$ plots were spread in the fourth quadrant along the second axes and the only species with a significant cover related the second axis, and consequently with the bryophyte cover, was *S. album.*

Table 5. 6 Summarize table of GLM performed on the species related with the first axis.

	Estimate	**F**	**p**
Bromus sterilis	-0,75	26.51	0,0001
Sedum sexangulare	-0,83	11.97	0,0008
Sanguisorba minor	-0,14	33.83	<0,0001
Carduus pycnocephalus	-0,11	10.23	0,001
Sedum hispanicum	0,74	77.46	<0,0001

Table 5.7 shows the results of multiple linear regressions of diversity variables with the scores of the first axis within clusters. Species richness displayed a significant negative correlation as well as Shannon index (Fig. 5.12). However, vegetation cover didn't show any relationship with this axis.
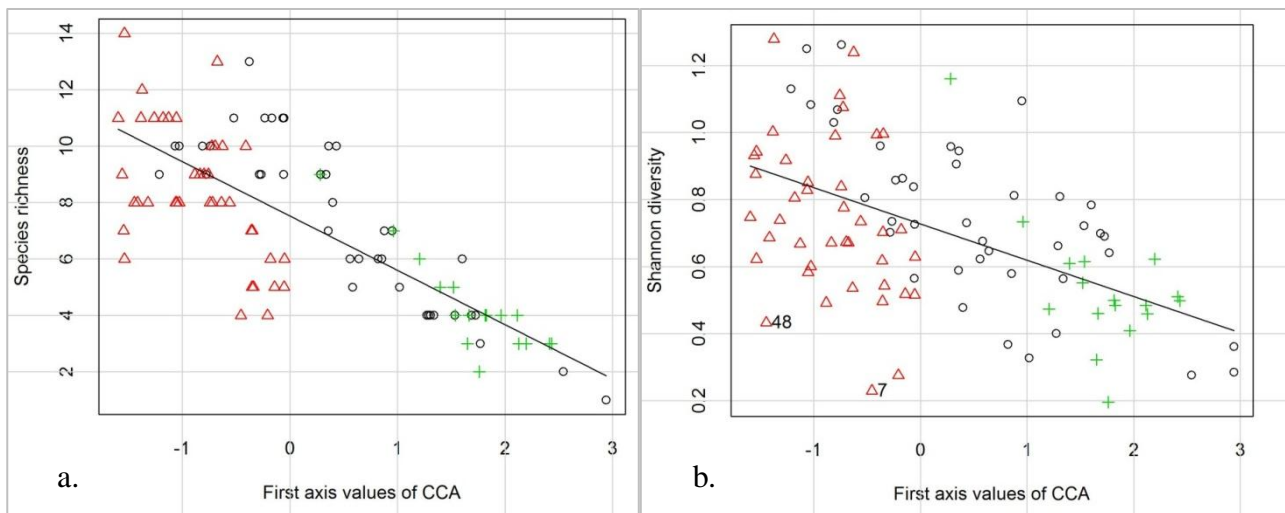
Figure 5. 13 Linear regression showing negative correlation between richness (a) and Shannon index (b) with the scores of first axis of CAA.

Table 5. 7 Multiple linear regressions of dependence variables with the scores of the first axis within cluster

Variables	Cluster	Estimate	t	Pr|t|
Richness (F=59,64; R²=0,65; p<0,0001)	GR1_Sa	-2,40	-9,25	**<0,0001**
	GR2	-0,96	-2,55	**0,01**
	GR1_Sh	-2,27	-8,04	**<0,0001**
Vegetation cover (F=1,82; R²=0,05; p=0,14)	GR1_Sa	-1,18	-0,58	0,56
	GR2	3,37	1,14	0,26
	GR1_Sh	3,22	1,46	0,15
Shannon (F=16,23; R²=0,33; p<0,0001)	GR1_Sa	-0,15	-5,19	**<0,0001**
	GR2	0,006	0,14	0,88
	GR1_Sh	-0,16	-4,92	**<0,0001**

6. Discussion

6.1. Diachronic discussion

The seasonal trend of species and vegetation pattern belonging to the "Rupicolous calcareous or basophilic grasslands of the *Alysso-Sedion albi*" were investigated at the high detailed scale in four fixed 1,5x1,5m macroplot in two gypsum outcrops of two SCI in Bologna province.

The maximum richness values reached in a single temporal step were about of 58% of the total amount of richness in a macroplot over the year. Anytime the sampling is carried out almost the half of all the annual richness could be not recorded. Concerning vascular plant, Dengler et al (2006) found on average 14(±5) species per 4 m^2 relevè in *Alysso – Sedion* vegetation of Central Europe during a single sampling in late spring. In the studied case were recorded 25 (±7) species in 9 temporal steps in 2,25 m^2 samples. The temporal heterogeneity within annual period and the dominance of annual species leaded to suppose the presence of a turnover in species pool between years as well as observed by Van der Maarel in the alvar of north Europe (1993). The high values of the turnover index between April 2012 and March 2013 seems to confirm this hypothesis. This would mean that assessing richness on gypsum outcrops communities may change considerably depending on the temporal resolution of the sampling.

As to sites, they showed a similar species pool centered mainly on *Sedum* genus and therophytes. Although differences in species composition among sampling units, similar trends in species richness, diversity and covers were shown. Autumn and early winter exhibited the peak in vegetation cover displaying a stable pool of species with some elements belonging to semi-arid grassland (i.e. *Geranium spp., Bromus sterilis, Trifolium* spp.). From late winter community presented an important change in species composition emphasizing the therophytes component. During the dry period the whole vegetation showed a drastic decrease in diversity, cover, richness and patchiness. Only few chamaephytes and hemicriptophytes (i.e. *S.album, S. sexangulare, Geranium rotundifolium* and *Carduus pycnocephalus*), maintained on average the 16% of the total diversity recorded in the year. In arid ecosystem the response of the species to the resource pulse could vary a lot between organisms causing the seasonal succession of species occurrence (Schwinning 2001, 2004, Chesson 2004). In this respect, July and August can be considered the zero point in a pulsating view of vegetation pattern of this habitat.

Differences in species appearance seem to be affected by both seasonal temperatures and rainfall which display a bimodal trend. Mean temperatures played an important role in leading richness and cover values explaining the 55% of the variability. Precipitations showing a significant positive

correlation with diversity, even though explained only about the 10% of total variability of annual diversity. Juhren (1956) and Baskin (1993) pointed out that annual plant species had a temperature-dependent germination, the patterns of which differ between species and it could explain stronger response of the habitat to this parameter. The main presence of therophyte in the present study may explain the main contribute of temperature. *Saxifraga tridactylites, Cerastium pumilum, S. hispanicum* and *Petrorhagia saxifraga*, for instance, endured only few weeks between March and May exploiting the first increasing in temperaturse and the earl annual peak in rainfall. These results support the theory according to which two species growing side-by-side and accessing the same source can avoid competition by being active during different times of the year (Cable 1969, Peláez et al. 1994, Golluscio et al. 1998, Nobel and Zhang 1997, Roupsard et al. 1999, Reynolds et al. 1999, Ogle 2004). This may increase the potentially number of species maintained in the habitat.

The significant correlation between bryophyte and vegetation over all the period, confirm the strategic role of mosses in this habitat (Lundholm 2006, Otsus and Zobel 2002). During the dry period, mineral and organic substrates tend to accumulate due to the little nutrient demand (Schwinning 2004).In this respect soil cover had an expected positive effect that increase from autumn due probably to the occurrence of those semi-arid grassland species that require greater availability of resource. For the same reason, bare rock decreases in suitability in the same period because of disappear of those species that found in this poor-nutrient substrate a free-competition space (i.e. *S. hispanicum and Saxifraga tridactylites*). However, the model considered a very small surface of the habitat and the conclusions carried out would have a demonstrative purpose, although in agreement with the result of other studies.

6.2. Synchronic discussion

The role of environmental heterogeneity in relationships with plant diversity was investigated in 99 plots randomly set. The sampled species represent about the 70% of all the species recorded for this habitat in Bologna SCIs and the 58% considering the outcrops of the whole Messinian gypsum bank recorded in the last inventory update (Life Gypsum 2011).

Species richness seems to be uncorrelated to total vegetation cover. Nowadays, no general consensus concerning a model describing the relationships between richness and productivity (commonly quantified by the biomass or vegetation cover) has emerged from theoretical considerations or empirical findings (Waide et al 1999). Several authors suggest that interspecific competition reduces species richness in productive habitats (Grime 1979, Grace 1999), but in

nutrient-poor environments this may be quite unimportant (Partel et al. 2000). Löbel (2006) founded in alvar grasslands a positive relationship between richness and vascular plant cover with a weak inversion only at high cover values. In the present study the lack of correlation may be due to the increasing of the *S.hispanicum* cover in rocky plots characterized by low richness. Indeed, in plots corresponding to more developed soil stages (Fig 5.10) the relationship became positive.

Low productivity may lead low competition among vascular plants in alvar grasslands (Huber 1994, 1999). Van den Maarel and Sykes (1993) explain this by the high tempo-spatial mobility of many short-lived alvar species allowing coexistence of a high number of ecologically equivalent species. Other authors pointed out the role of microsite heterogeneity in maintain diversity in plant communities, (Ricklefs 1977, Grime 1979, Tilman 1982, Tilman and Pacala 1993, Palmer 1994, Lundholm 2003 and 2006).

Species richness and diversity were positively affected by an increase of substrate heterogeneity. Since plant species differ in their germination and establishment responses to different kinds of substrates (Harper et al. 1965, Grubb 1977, Silvertown 1981, Zamfir 2000), plots with higher substrate diversity might be predicted to contain safe sites for many species (Gigon and Leutert 1996). However, the response of richness to substrate heterogeneity may depend by the habitat limiting resources and physical features: the fewer the limiting resources are the lower the heterogeneity effect (Tilman 1993). Furthermore, Van der Maarel (1995) pointed out that in small scale plot ($\leqslant 0{,}25$ cm^2) plants size plays an important role in determine richness. In this study the relationship between richness and substrate heterogeneity depended also on the relative cover of the different life form in the plot. For instance increasing in hemicryptophytes cover promoted richness only in plots with few substrate typologies while in the same situation, the increasing in therophyte leaded a decreasing in richness values.

In addition, plots characterized by deeper and homogeneous soil cover or litter, may become more attractive for species with low mobility, large size and longer life cycle respect to more heterogeneous but low productive substrate. This could be due to the size of bare rock, bryophyte cover and smaller soil patches. On the other hand annual species with high mobility (sensu Van der Maarel 1993) and small size, as therophytes, may contribute in increase plant richness in heterogeneous plots by exploiting empty microsites avoiding competition.

Species composition seemed to vary according with the gradient of soil development. The first axis of constrained ordination (Fig. 5.10) represents a theoretical variable that may explain this gradient while the second one highlighted the negative relationship between bryophyte mat and litter.

Nevertheless, the variance explained by the constrained ordination was not high. Other studies using similar approaches indicate that there is a consistent amount of stochastic variation in the distribution of species (Chiarucci et al. 2001). However it was possible to point out the strategic role of *S.hispanicum* in the earlier stage of habitat dynamics and its tendency to remain isolated form the other species. This result confirmed the observation of Ferrari (1974) who described the association *Cladonio-Sedetum hispanici* for the gypsum heat cliffs, with *S. hispanicum* and *Cladonia convoluta, Bryum bicolor* and *Barbula convoluta* as a "core characteristic" species. Dengler et al (2006) extended the importance of this association in the chalky alvar of central and north Europe. On the other hand species such as *Bromus sterilis* and *Carduus pycnocephalus* have greater cover in more developed situation and might lead the community in situation closer to semi-arid grasslands of *Festuco –Brometea*.

The second direction followed by the species colonization was represented by the bryophyte mat. Several studies underlined the correlation of the cryptogame component as facilitator in vascular colonization of outcrops (Lundholm 2006) and basophilus grassland in general (Otsus and Zobel 2002, Keizer 1985). During our synchronic survey the only weak relationship with bryophyte cover was found for *S. album*. However the detection of the effect of moss may depend on the sampling scale. In plot of 5x5 cm Mandolfo (2013) remarked the importance of small bryophyte patches as germination microsite for *S.hispanicum* as strategic refuge from competition.

Topographic features were indirectly correlated with diversity by affecting other parameters such soil depth (greater in concave one). Generally microtopography, which affects the soil evolution and availability, is an important factor in high limited resource habitats such in cliff faces and rocky outcrops (Lundholm 2006, Kuntz 2006, Medina 2006). However, our field observation leaded to hypothesize that micro-scale topography, at 1-5 centimeter scale, might offer the best information about species establishment.

7. Conclusion

Plant species diversity in rupicolous gypsum grasslands of the *Alysso-Sedion albi* is a complex phenomenon, influenced by many abiotic and biotic environmental factors. The responses of diversity pattern depend both on the resources availability, environmental heterogeneity and the manner in which the different taxonomic groups access to them during the year. This vegetation may, in conclusion, be described by the coexistence of three species groups.

The first group is dominated by *S. hispanicum* displaying low richness, due to the homogeneity and lack of resources of substrate according with the well-known relationship between species richness and substrate heterogeneity (Tilman 1993). Species with small size and rapid life cycle are favored. It is due to their ability to exploit small fissure and micro patch of bryophyte that hold little quantity of nutrient and water. In addition, this group is associated with the early rainfall events in the year, appearance and persistence from February to April avoiding the warmest months.

The second group is leaded by *S. album* and shows intermediate richness values. It was often, but not exclusively, found on larger bryophyte patches generally completely occupied. Such fact is due to the reptant life form of this species. However its increase in cover is slow and limited to the spring months and hardly present a large coverage of the plots.

The third group has the highest species richness and corresponds to the highest substrate heterogeneity. It contains species with the widest ecological range, which requires stable resources for the longest period of time (from October to May). This group is associated also with the deepest soils, although their depths never exceed 5 cm.

It can be, finally, found a gradient from early colonization stages, characterized by greater slope and predominance of bare rock, to the more developed soils. The high level of diversity generally recorded in this habitat become even more important if considered during the whole year and might grow even through the years due to the high mobility of the species.

The low frequency of occurrence of the most species considered makes hard to gather information about their ecological behaviour. In addition, it needs to take into account the different scale to which the species live, their relationships with environment and their interaction with the surrounding species. Results suggest to set up targeted studies adapting the sampling scale on the entities of interest (Mandolfo 2013, Csergő 2008).

Image data analysis and photoplots may help in this respect. From images it is possible to consider from centimeters to meters in the same sample. Furthermore images could be archived and quickly

recovered for further analysis. The demonstrative GLMM carried out on vegetation spatial-temporal could be adapted also to analyze the spatial-temporal patterns of species populations. The main contribution of the proposed model is to provide a new approach to the study, in this habitat, of the relationship with substrate conditions that considers simultaneously also its temporal change in a continuous and high detailed framework. However, it needs larger samples to generate reliable outputs.

In the next future researches should be addressed to investigate a multi scale assessment of species richness in this habitat. It will be possible to understand how the small surfaces affect plant diversity and which are the best monitoring scales. For this purpose, the Landscape Ecology approach applied to the micro-landscapes based on ground photography could represent a useful tools reducing considerably the sampling time. Moreover, a long term study on the colonization pattern of bare rock might be useful in predicting vegetation changes as well as the species turnover rate. Moreover such analyses might be useful for improve active restoring management plans.

In conclusion, the rupicolous calcareous or basophilic grasslands of the *Alysso-Sedion albi* offer great opportunities for the ecological evaluation of plant diversity in an arid habitat and in the meantime for their management and conservation. Studies on plant diversity in dry grassland ecology are carried out for several years in Central and Northern Europe. In Italy this is one of the few attempts to understand such environmental features and to formulate guidelines applicable to a larger range of dry grassland types, especially when they are "ecological islands" as in the Northern Apennines.

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