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Species and functional composition of some abandoned fields in the Northern Apennines (Italy). A detailed overview with particular reference to the Habitat 6210 in the EU 92/43 Directive.

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Key words abbreviations

- AWC Available Water Capacity
- CCA Canonical Correspondence Analysis
- CVRE Cross-Validation Relative Error
- CWM Community Weight Mean
- DCA Detrended Correspondence Analysis
- EIV 's Ellenberg's indicator value
- FD Functional Diversity
- FDiv Funcitonal divergence
- FEve Functional Evenness
- FRic Functional Richness
- G1 group 1
- G2 group 2
- G3 group 3
- ISA Indicator Species Analysis
- M soil moisture
- MRT Multivariate Regression Tree
- N soil nutrients content
- R soil chemical reaction
- RDA Redundancy Detrended Analysis
- RLQ Fourth Corner analysis
- SBT social behaviour type
- T air temperature

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Research structure

This work is organized as follow: in the first four chapters we try to explain the status of the vegetation analizing species composition and ecological aspects of the communities and in the last chapter we focus in particular on their functional assemblage and the direction of the succession already emerged from the species composition.

In details Chapter 1 is an introduction of the European scenery on the scientific matter of the research, as well as on the aim of my work and about its geographic location.

Chapter 2 describes how different macro and micro-scale environmental factors affect species richness, as well as the ecological requirements of the communities according to Ellenberg's values.

Chapter 3 contains a plant species assessment through the Indicator Species Analysis (ISA) and the description of species behaviour including each species into own Social Behaviour Type (SBTs hereafter) following their phytosociological preferences in higher syntaxa (class, order)

Chapter 4 describes the life strategies adopted by species to occupy the surronding space (morphological traits) and their life forms.

In Chapter 5 two of the communities that are closer the standards of semi-natural grasslands indicated in the 92/43/EEC Directive, are described by a functional approach at different levels (functional traits and functional diversity).

Introduction

Semi-natural grasslands (6210 code 92/43/EEC Directive) are among the speciesrichest plant communities in Europe in terms of the number of plant species they support per unit area. Owing to their anthropogenic origin these grasslands correspond to a wide ecological and vegetation diversity. All over Europe, since the beginning of the 20th century, the abandonment of the traditional management practices in agriculture had led to the loss of several semi-natural grasslands. The result has been a loss of biodiversity and total area of grassland in the EU fell by an average of 12% between 1975 and 1998. The absence of grazing and cutting or both has produced a continuous decrease of many species that depend by such practices, as many orchids species which give a conservation priority character (6210*) to this habitat (EC 2007a). In Italy the semi-natural grasslands cover the 42.96% of the Nature 2000 network.

They prevailing lie in the Alps (LASEN 1989, FEOLI CHIAPPELLA & POLDINI 1993, CERABOLINI 1996, FRISINGHELLI et al. 1996) and in the central Apennines (BIONDI et al. 1995, MATT 2003). They are undergoing to a progressive qualitative and quantitative regression because of different threats in different sites. The overgrazing with the nibbling, trampling and poaching cause soil erosion and a decrease in species richness while the under grazing lead to the spread of the false brome, i.e. *Brachypodium rupestre*, a competitive tall grass linked to the soil increased availability of nitrogen. Indeed, the effect of nitrogen enrichment results in a strong reduction in species diversity and in a change in the vertical structure of the grassland vegetation caused by an enhanced growth of more competitive 'tall' grasses, especially of stress-tolerant species, which have a slightly higher potential growth rate and more efficient nitrogen utilization (WERNER & SPRANGER 2000).

The abandonment of agro-pastoral activities results in turn in the establishment of scrubs. This vegetation dynamic stage can correspond to an increase of soil nutrients with a decline of species richness (BUGLIFE 2007) in a short time.

Also, climatic change seems to be a future threat for this kind of grassland. In the early succession they contain fast-growing or short-lived species that are more likely to be affected by this phenomenon than older calcareous grasslands. In addition, other local factors affected this habitat as inappropriate human activities, alien plants invasion and fires. Troiani N. Species and functional composition of some abandoned fields in the Northern Apennines (Italy). A detailed overview with particular reference to the Habitat 6210 in the EU 92/43 Directive.CicloXVIII, May 2016

In Italy more than 70% of high nature value (HNV: ANDERSEN et al. 2003) farmland is associated with permanent grasslands (TRISORIO & BORLIZZI 2011) and the main causal factor of changes in botanical composition and a decrease of biodiversity in grasslands (MARINI et al. 2008; TARGETTI et al. 2010) is the widespread abandonment of traditional practices. Several previous studies about grassland abandonment are provided. With reference to the Apennines the information supplied by the "Italian Interpretation Manual of Habitats of the 92/43/EEC Directive" (BIONDI et al. 2009) about the 6210 habitat are chiefly for semi-natural grasslands on argillaceous and /or arenaceous substrates. Some local phytosociological studies (TONIOLI et al. 2004, BIONDI et al 2014, BIONDI & PESARESI 2004, BIONDI et al. 2001, 2005) are available. Studies related to the effects of management and /or abandonment of these semi-natural grasslands are provided for the Northern as well as for the Central Apennines. Some works about plant functional traits are available for the Northern Apennines but exclusively regarding the leaves functional traits (TARGETTI et al. 2013). Studies about the grazing values of the semi-natural grasslands on the hilly landscape of the Northern Apennines were performed in the past (TONIOLI et al. 2004). Information about functional traits are related only to few species as Bromus erectus, Brachypodium rupestre, Dactlylis glomerata and Holcus lanatus (BOLZAN 2008).

This cultural scenery justify the need of our study. In a protected area of the Northern Apennines we have considered 1) the status of the grasslands which are locally referred to the 6210 habitat (DELL'AQUILA et al. 2001-2003) with reference to their species composition and vegetation diversity and 2) the effect of the abandonment on the diversity of plant functional strategies. The functional diversity approach is considered an important descriptor of processes which lead to a species composition (PAKEMAN 2011).

This work tries also to answer to the aims of Natura 2000 Network and of the European Agriculture Policy to preserve the 6210 habitat (sensu 92/43/EEC Directive). The habitat protection requires before its properly definition as well as its conservation status assessment (EJRNÆS et al.2004).

We focused our work on abandoned grasslands belong to Natura 2000 Network. These grasslands are abandoned for 40 years. Their restoration needs an appropriate management measures to avoid the vegetation changes due to the natural succession towards forest. Currently, the restoration of grassland on former croplands is a high priority of nature conservation (STADLER 2007) and is one of the most frequent habitat restoration actions in central and northern Europe (CRAMER et al. 2008).

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Indeed with proper management, abandoned areas could be viewed as new "scattered elements" in areas where forest recovery and expansion are underway. In agricultural landscapes, which are generally undergoing processes of intensified land use, grasslands provide different benefits for environment, concerning biodiversity (birds, insects) and local economy (production, employment) as well as their landscape aesthetic value.

This study tries to clarify the potentiality of the abandoned fields to lead to different semi-natural grassland under different environmental conditions. The species composition of such grasslands depends on factors as resource availability (EJRNÆS et al., 2008), time since abandonment, and availability of seeds in neighbouring habitats (GIBSON and BROWN 1991, PYWELL et al. 2002, RUPRECHT 2006), altitude and land forms (BURRASCANO et al. 2013), soil features (CATORCI and GATTI 2010), land use history (CATORCI et al. 2011a) and disturbance type (PECO et al. 2006; de BELLO et al. 2007; CATORCI et al. 2012; RIBEIRO et al., 2012; BAGELLA et al.2013).

We analysed several of these features to verify the conservation status of the vegetation communities classified as habitat 6210 in the Nature 2000 maps supplied by the Emilia-Romagna Region (<u>http://ambiente.regione.emilia-romagna.it</u>) and if they correspond to the standards of the Annex I of the Habitat Directive. The vegetation features we have evaluated could be useful to predict the natural changes of the grasslands and to apply appropriate economic resources for their management.

1 Semi-natural grasslands scenery

In the Northern Apennines - as overall temperate Europe - many abandoned fields from 40 years are covered by semi-natural grasslands, sensu WESTHOFF (1983). At early succession stage they are comparatively poorer of species (MARTEINSDÓTTIR & ERIKSSON 2014) than sub natural and natural grasslands. These plant communities characterize the hilly and mountain landscapes in the most of Europe (PERONI et al. 2000). They represent important scattered elements in landscapes generally under wide processes of land use changes, where forest recovery and expansion are acting. As is known, natural and socio-economic processes are threatening the biodiversity leading to homogeneous landscapes (POSCHLOD et al. 2005, PREVOSTO et al. 2011), thus the conservation and management of these open habitats will be key elements within the environmental diversity (ROUNSEVELL et al. 2005, RIEGER et al. 2000). Actually, the restoration of grassland on former croplands is a high priority of nature conservation (STADLER 2007) and is one of the most frequently applied habitat restoration actions in central and north Europe (CRAMER & HOBBS 2008), following the European Union 92/43/EEC Directive for the protection of priority European habitats. A proper definition of target habitats is therefore essential for the implementation of habitat protection programs (EJRNÆS et al. 2004) as well as standardized and validated approaches aimed to the assessment of the conservation status (DE BELLO et al. 2010).

Starting from the second half of the twentieth century, land use policies in Europe have been leading to the abandonment of extensive farming activities (LAVOREL et al. 1998), which led to succession changes toward shrub lands and woodlands (POSCHLOD and WALLISDEVRIES, 2002) and to a wide loss of their extension (PREVOSTO et al. 2011). Post-cultural herbaceous communities are particularly interesting from a floristic viewpoint and few information about them are available in the northern Apennines. These mountains lie in a geographic transitional zone between temperate continental and temperate oceanic macrobioclimates (RIVAS MARTINEZ et al. 2011 PESARESI et al. 2014), and are characterized by clastic and terraced clastic sedimentary lithological substrates (SMIRAGLIA et al. 2013) different by those structuring the remnant calcareous and arenaceous Apennine chain. The northern Apennine post cultural herbaceous communities are placed both on the mineral eroded soils which lie on badland ("calanchi") steep slopes and on flatter and compact sandy substrates with deep and narrow cutting (BOLZAN 2008). This ecological diversity creates biological scenery. which Apennine is peculiar of this geographic sector

1.1 Objectives

Without management practises, the vegetation processes go ahead hindering the conservation of plant communities which are referred to the 6210 habitat.

The main purpose of this work was to understand how abandoned old fields develop into seminatural grasslands belonging to either 6210 habitat according to Nature 2000 criteria.

The results might contribute to choose the best management practices of the habitats in the study area in order to preserve their biodiversity.

1.2 Study area

The study was performed in the following five sites of the Natura 2000 Network :

-IT4050001 Gessi Bolognesi - Calanchi dell'Abbadessa

-IT4050014 Monte Radicchio - Rupe di Calvenzano

-IT4050012 Contrafforte Pliocenico

-IT4050003 Monte Sole

-IT4050011 Media Valle del Sillaro

The sites are located in the Northern Apennine hilly landscape, which is characterized by chalk and sandstone outcrops (centroid coordinates: 11° 18' 20.6" E; 44° 20' 52.4" N - Figure 1).

Their altitude range is from 200 to 500 m a. s. l. Local climate shows a mean annual temperature of 13-14 °C with 9 °C as mean minimum and 18 °C as mean maximum. The annual rainfall is about 800 - 900 mm. July is the driest month (average rainfall 40-50 mm), and winter cold stress occurs between early December and late February. From a bioclimatic point of view, the area is included between continental sub-Mediterranean and oceanic sub-Mediterranean bioclimatic belts, upper mesotemperate thermotype and upper subhumid ombrotype (PESARESI et al 2014).

Soils are generally deep (30-60 cm) with basic pH (7.0-8.0) and, mainly, with sandy and silty clay textures.

The grassland vegetation of the abandoned old fields belongs to different phytosociological taxa. According to BIONDI et al (2005) it can be included in *Festuco-Brometea* class, *Brometalia erecti* order. A detailed study by UBALDI (2013) included such vegetation into *Agropyretea repentis* class, *Artemisio albae-Brometalia* order, *Convolvulo-Agropyrion* alliance, The actual natural potential vegetation is referred to *Quercus pubescens s.l.* woods of the *Peucedano cervariae-Quercetum pubescentis* association and hop-hornbeam woods of the *Ostryo-Aceretum opulifolii* association (BLASI 2010).

Scattered shrubs and trees as *Rubus ulmifolius, Rubus caesius, Rosa canina, Spartium junceum, Fraxinus ornus* can be seen only in the sites which seems to be furthest from semi-natural conditions. Here the grasslands are dominated by *Bromus erectus* and belong to *Mesobromion* alliance. Several species of orchids as *Orchis morio, Orchis coriophora, Ophrys fusca*, *Anacamptyis pyramidalis* can be found.

The nearness of these semi-natural grasslands to a more natural status is also underlined by shrubs and trees on the border (Figures 1.1-1.2).



Figure 1: The distribution of the sites of the Natura 2000 network considered. The sites are in the Northern Apennine belonging to the Bologna province (centroid coordinates: 11° 18' 20.6" E; 44° 20' 52.4" N)



Figure 1. 1: An abandoned old field where shrubs and An abandoned old field where shrubs and small trees are establishing (IT4050011 Media Valle del Sillaro).



Figure 1. 2: A grasslands from an abandoned old field on a compact sandy soil. Shrubs and trees are distributed on its border (IT4050003 Monte Sole).

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2 Ecological groups and species diversity

The characterization of environmental driving forces that influence plant composition and the understanding of species changes due to different management strategies, plays an essential role for grassland biodiversity conservation (HODGSON & ILLIUS 1996). Such an analysis can be based on the phytosociological characterization of vegetation, since the phytosociological characterization of a plant community reveal both its ecological and dynamic conditions It is well known that is the quantitative relationships between species belonging to different syntaxonomic units as classes, orders, alliance, can reveal the dynamic processes which are prevailing in the community (BIONDI 2011).

Environmental and ecological parameters indicated by Ellenberg's values (ELLENBERG et al. 1991) can be considered crucial for characterize ecological features starting from the species composition: altitude and land forms (BURRASCANO et al. 2013), soil features (CATORCI and GATTI 2010), land use history (CATORCI et al. 2011 a), disturbance type (DE BELLO et al. 2007, PECO et al.2006, CATORCI et al. 2012, BAGELLA et al. 2013, RIBEIRO et al. 2012) and timing (CATORCI et al. 2014 a). Ellenberg's values, although are indirect measures and mutually correlated (CHYTRY' et al. 2009) are widely used for the assessment of site features, their habitat relationships (DIEKMANN 2003, WASOF et al. 2013, SAMMUL et al. 2008 b) and for the prediction of likely successional stages.

It is well known that species or vegetation spatial patterns are usually too complex to be simplified in either strictly geographic terms or strictly edaphic or local topoclimatic ones (TZONEV et al. 2006).

Often these patterns are scale-dependent. They show stronger edaphic effects at finer scales, while there are stronger geographic differences at broader scales (CHYTRY[´] et al. 2002; KUZELOVA[´]& CHYTRY[´] 2004) However, for many wide-scale data sets, edaphic, local topoclimatic, and geographical factors can have comparable importance and interact in complex ways to form a vegetation pattern (BERGMEIER & DIMOPOULOS 2001; KNOLLOVA[´] & CHYTRY[´] 2004).

These statements are particularly pertinent in submediterranean mountains (RIVAS-MARTINEZ AND RIVAS- SAENZ 1996–2009) where altitude, slope aspect and angle, besides soil micro-scale features (e.g. soil depth and texture), are key factors in determining the intensity and length of drought and cold stresses (SOMOT et al. 2008, PESARESI et al. 2014), thus of the spatial distribution and the species composition of plant communities. Although in the abandoned anthropogenic plant communities the relationship between species richness and

environmental factors are generally still scarce (MERUNKOVÁ an

CHYTRÝ 2012), the assessment of how biotic and abiotic features affect their species composition is a key step in a conservative management of these systems.

To analyze the factors which affect the distribution of plant communities and their species composition, we selected the following macro-environmental features:

-altitude (m a.s.l.)

-slope aspect (azimuth degrees)

-slope angle (vertical degrees)

These features shape the distribution of potential vegetation types in temperate regions at the landscape level (BLASI et al. 2000), and have been used to build models of the realized ecological niche of a species (e.g. CHOLER & MICHALET 2002).

Moreover, we used some fine-scale features as

-outcropping rock cover (%)

-litter cover (%)

-soil available water content (AWC)

They affect the species composition of the grassland plant communities (CATORCI et al 2012, LAVOREL et al. 1998, GALVANEK and LEPS 2011, JANISOVA et al. 2013).

2.1 Materials and methods

2.1.1 Sampling design and data collection

We collected data in June-July 2013 in order to observe both the spring and the summer flowering species. Using a GIS, we overlaid the study area with a grid composed of 100 x 100 m macro-plots, each further divided into 100 plots (10 x 10 m). In each macro-plot we randomly selected one 10 x 10 m plot, excluding those that fall completely or partly on woods, brush and shrub vegetation, as well as those that fall in a buffer of 50 m from these vegetation types to avoid the edge effect. In each plot we collected data on altitude (m a. s. l.), slope aspect (azimuth degrees), slope angle (vertical degrees) and litter coverage (%) as well. In all we surveyed 100 plots. In each plot we recorded species cover values (percent values, visually estimated), altitude (m a.s.l.), slope aspect (azimuth degrees), slope angle (vertical degrees), outcropping rock cover and litter cover (percent values, visually estimated), soil depth (cm, five measurements per plot) and collected five soil sub-samples, taken within a depth of 30 cm and combined in one bulked sample.

Climatic data were gathered from RAPE database (Regional Agency for Prevention and Environment). The climatic data for each plot are from the closest meteorological station with the same altitude. In detail:

-San Luca 286 m a. s. l and Settefonti 336 m a. s. l. for IT4050001 Gessi Bolognesi - Calanchi dell'Abbadessa

-Sasso Marconi 275 m a. s. l. for IT4050003 Monte Sole and IT4050012 Contrafforte Pliocenico

-Monzuno 620 m a. s. l. and San Clemente177 m a.s.l. for IT4050011 Media Valle del Sillaro

To characterize the plant communities from an ecological point of view we used the Ellenberg bioindication values (EIVs) (T - temperature, U - soil moisture or water availability, R - soil chemical reaction) according to Ellenberg et al. (1992).

Species nomenclature follows Conti et al. (2005).

2.2 Data treatment

Following WARREN (2008), aspect azimuth was firstly converted from the 0-360 compass scale to a linear (0-180) scale. To the northern exposure a value approaching 0 and to the southern exposure a value approaching 180 were given. This transformation also converted East and West azimuth degrees so that they were equally distant from North. Moreover, as south-south-west-facing slopes are the warmest exposures, the azimuth was shifted to a minimum on north-north-east facing slopes (22.5°) and a maximum on south-south-west facing Data on soil depth were averaged for each plot. Soil samples were analysed in the laboratory of the University of Bologna to determine the percentages of skeleton, sand, loam, and clay. In order to have a proxy for the soil water regimes, for each plot we calculated the soil Available Water Capacity (mm, hereafter AWC) using the software developed in Microsoft Office Excel 2000 by ARMIRAGLIO et al. (2003). AWC represents the maximum amount of available water the soil can provide. It mainly depends on soil texture and depth and, secondly, on soil specific weight and organic matter content (MCRAE 1991). The input variables processed were soil depth (cm), soil texture (percentage of sand, clay, loam, and percentage of skeleton), and a coefficient of water available for plants under a pressure of 0.05-15.00 bars, obtained from tabulated values (MCRAE 1991). Even though the outputs obtained are imprecise because they lack data on organic matter, nonetheless they offer a good proxy for comparing plots, because the estimation was based on soil depth and texture, and thus was independent from vegetation structure (i.e. vegetation cover, tiller density, canopy height, litter decomposition rate, etc.), which might alter the soil organic matter content.

2.3 Data analysis

To identify homogeneous groups of relevés and to assess the influence of macroenvironmental variables on species composition a constrained clustering using Multivariate regression tree analysis (MRT, DE'ARTH 2002) with 100 iterations and 29 cross-validation groups was executed on two matrices, namely "species x relevés" and "environmental variables (altitude, slope aspect, slope angle, AWC, outcropping rock cover, and litter cover) x relevés". Prior to MRT analysis, species data have been transformed using chord transformation (LEGENDRE & GALLAGHER, 2001) which expresses the data as relative abundances per sites. This removes from the data the total abundance per site, which is the response of the species to the total productivity of the sites (BORCARD et al. 2011). An overall error statistic (Cross-Validation Relative Error, CVRE) was computed for each test group and partition size (number of groups), to choose the optimal size of the tree. Cross-validation is a re-sampling analysis repeated a number of times for successive and independent divisions of the data (in this case, species cover) into random test groups of objects (relevés) (LEGENDRE & LEGENDRE 2012). CVRE varies from zero, for a perfect set of predictors chosen for the splits of a tree, to close to one for poor predictors. For each partition size, the mean and standard error of all CVRE estimates was computed. Generally, the smallest tree (i.e. a tree with fewer splits) within one standard error of the smallest CVRE value is selected (BREIMAN et al. 1984), but one may select the tree that has the smallest CVRE value, as well (LEGENDRE & LEGENDRE 2012).

To provide a general overview of the ecological features corresponding to the plant communities highlighted by MRT, we calculated mean, standard deviation, 1st and 3rd quartiles for each environmental variable. We used the Shapiro-Wilk test (ROYSTON 1995) to test the distribution normality of data. As data were not normally distributed, we ran the non-parametric Wilcoxon Exact Rank test to evaluate differences between groups at each step of clustering.

Following the indication of ZELENY'& SCHAFFERS (2012), for assessing the environmental features characterizing the MRT groups of relevés a modified permutation test using mean randomized EIVs (T, U, R) in a Detrended Correspondence Analysis (DCA) was performed. We calculated mean and median values of EIVs for each group of relevés. Bioindication values were gathered from PIGNATTI (2005) and GUARINO et al. (2012).

The differences in species richness between groups of relevés, was evaluated by a rarefaction analysis. This was performed on the same matrices processed for MRT analysis. For group the rarefaction curve was calculated by repeatedly re-sampling the pools of N samples, at random, plotting the average number of species represented by 1, 2, ..., N samples. Sampling was done without replacement, within each re-sampling. In such a way, rarefaction generates the expected number of species in a small collection of n samples drawn at random from the large pool of N samples (KOBAYASHI 1974, GOTELLI and COLWELL 2001). Moreover, for each group of relevés we assessed species diversity calculating Shannon-Wiener (H') and Gini-Simpson (S) diversity indices, together with Shannon evenness (E).

To perform MRT analysis, Shapiro-Wilk tests, Wilcoxon exact rank tests, and DCA we used the R software packages mypart version 1.6-1 (mypart function), stats version 2.15.2

(shapiro.test function), exactRankTests version 0.8-27 (wilcox.exact function), and vegan version 2.0-10 (decorana function), respectively (version 2.15.2, R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org). The R function used for projection of mean Ellenberg indicator values onto an ordination, with modified permutation test was envfit.iv (ZELENÝ and SCHAFFERS 2012).

2.4 Results

2.4.1 Ecological groups

MRT analysis generated a 3-leaf MRT with CVRE = 0.88 and standard error =0.055 (Figure 2). This result was selected as the best solution during the cross-validation iterations. Table 2 shows the mean, standard deviation and statistical significance of the environmental variables between groups highlighted by MRT in the 3-leaf tree.

The rockiness is the variable discriminating between the two branches in the first node of the 3leaved MRT. The relevés of the third leaf (G3) are characterized by the presence of outcropping rocks, while in the other groups rockiness was absent. Moreover, litter cover (higher values), slope angle (higher values), altitude (higher values) and AWC (lower values) showed significant differences among relevés of G3 and G1+G2 (Table 2). The second partition was defined by altitude (Figure 2). Group G1 includes relevés located at an altitude higher than 290 m (mean value 418 m a.s.l.), with low slopes angle, while lower altitude, slightly lower AWC values and lower litter coverage characterized relevés belonging to group G2 (Table 2).

The first two components of DCA based on mean randomized EIVs value and "releves x species" matrix (eigenvalues 0,52 and 0,25) were related to soil chemical reaction (R) and temperature (T) respectively. Temperature was the main factor ($r^2=0,67$, P = 0,02), that discerned the three different groups highlighted by MRT that were recognized also in DCA scatter plot (Figure 2.1).

The differences in mean and median values of EIVs comply with DCA results (Table 2.1). G1 group positively correlated with EIV soil reaction (R), and characterized by mean R-value higher than G2 and G3 groups. G2 group, positively correlated with EIV temperature (T), was characterized by mean T value higher than G3 and G1 groups. Moisture values don't show differences between the three communities (Table 2.1).

2.4.2 Species diversity

209 species were sampled. The values of richness, mean richness, the Shannon-Wiener (H'), Evenness and the Gini-Simpson (S) diversity indices are shown by Table 2.2.

Figure 2.2 shows the rarefaction curves for each group of 3-leaf MRT. The curve of G1 show a higher richness values with its slope that differ from the other two groups whose slopes were more similar. The three curves do not reach a plateau. Particularly, G2 group contains few plots and the trend of its rarefaction curve cannot to be define exactly. In Table 2.2 G1 group was richer in species while G3group shows the highest H and S values.



Figure 2: The 3-leaved multivariate regression tree for the species data set, constrained by the explanatory variables data set. The 3-leaved multivariate regression tree for the species data set, costrained by the explanatory variables data set. The threshold values shown for each partition of the tree correspond to the mean of the two limit values of the considered variables at the break between the branches. The relative abundances of the species are shown in histograms at the tips of the branches. Under each histogram, the sum of squared errors for the group and the number of relevés in the leaf (n) are indicated. Below the tree the residual error (Error), the cross-validation error (CV Error) and standard error (SE) are indicated.

Table 2: Descriptive statistics of the environmental variables for the groups highlighted by the Multivariate regression tree analysis and statistical significance of differences, from Wilcoxon Exact Rank test, between the groups (SD: standard deviation; *Significance codes : P < 0.05; ** P < 0.01; *** P < 0.001; n.s.: not significant).

Group	Statistics	Rockines	Litter	Slope	Slope	Altitude	AWC
		s (%)	(%)	aspect (°)	angle (°)	(m a.s.l.)	(mm)
G1	mean	0.00	24.30	129.0 0	14.40	418.17	108.80
	SD	0.00	32.60	46.80	6.46	56.50	4.06
	1 st quartile	0.00	0.00	67.50	10.00	384.00	105.92
	3 rd quartile	0.00	30.00	157.5 0	20.00	460.00	110.00
G2	mean	0.00	8.00	155.2 5	18.00	205.80	107.90
	SD	0.00	16.80	7.10	7.80	43.30	0.55
	1 st quartile	0.00	0.00	157.5 0	15.00	201.00	107.60
	3 rd quartile	0.00	0.00	157.5 0	25.00	238.50	107.80
G3	mean	14.40	53.10	143.0 0	19.90	474.70	104.06
	SD	14.40	28.02	24.05	6.70	56.20	6.10
	1 st quartile	5.00	30.00	112.5 0	15.00	454.00	100.96
	3 rd quartile	20.00	80.00	157.5 0	25.00	513.00	108.80
G1-G2	vs G3	***	***	n.s.	***	***	***
G1 vs (52	***	***	n.s.	n.s.	***	**



Figure 2.1: Scatterplot of the Detrended Correspondence Analysis (DCA) ordination executed on mean randomized EIVs and relevés data set. Eigenvalues: axis 1=0,52, axis 2=0,25; 77% of the total variance explained by the first two axes. T-temperature, R-soil chemical reaction: circles - G1; triangles - G2; crosses - G3. Only significant vectors (P < 0.05) have been represented.

Table 2.1: Mean and median Ellenberg indicator values (EIVs) for air temperature, soil moisture and soil reaction of each group (G1, G2, and G3) emerging from the multivariate regression tree analysis.

	Group					
	G1		G2		G3	
	Mean	median	Mean	median	mean	median
EIV for air temperature	5.98	5.68	7.72	7.67	6.92	6.93
EIV for soil moisture	3.58	3.44	3.45	3.56	3.47	3.46
EIV for soil reaction	7.05	7.28	5.64	5.67	5.68	5.75



Figure 2.2: Rarefaction curves for species richness, calculated through re-sampling of $10 \ge 10$ m plots Randomized species number is plotted against the sampling intensity for each group of 3-leaved Multivariate regression tree. Error bars indicate standard deviation.

Table 2.2: Details on the groups highlighted by the Multivariate regression tree analysis (G1, G2, and G3). Total and mean richness and values of Shannon-Wiener (H'), Shannon Evenness (E), and Gini-Simpson (S) diversity indices are shown.

Group	Total richness	Mean richness	Η'	Е	S
G1	173	27.36	2.94	0.57	0.84
G2	91	26.90	2.86	0.63	0.89
G3	135	29.11	3.14	0.64	0.90
-					

2.5 Discussion

MRT analysis highlighted three main ecological groups (Figure 2), linked to different environmental features (Table 2). These features are due to different soil water regimes, and different ecological requirements as indicated by Ellenberg's indicator values (Table 2.1). This is consistent with previous researches highlighting how in sub-mediterranean climates, landforms are key factors in determining the plant communities distribution and composition (AREVALO J.R. et al. 2012). The main ecological factor behind these variables is the total solar radiation amount per unit area (BIONDI et al. 2011). In fact, on south-facing slopes, the greater radiation in summer dramatically reduces the soil water content (JOFFRE and RAMBAL 1993), posing one more stress factor faced by plants in mountain areas (CATORCI et al. 2014b)

In details, G1 group represents the community with the highest species richness and the lowest evenness values probably highlighting homogeneous fine-scale conditions (Table 2). This community shows a positive correlation with high AWC (Table 2), low EIVs temperature and high EIVs ph (Table 2.1). Actually, it developed in mesophylous conditions mainly due to absence of rockiness and and to slope not too steep.

G2 group has low species diversity (Table 2.2) and occurs at the lowest altitude and on south facing aspect (Table 2). It shows too harsh condition to develop (highest EIVs temperature) into dry hay meadows (that usually do not host species of *Thero-Brachipodietea*) and morphological features as semi-flat slopes and deep soils of sites does not seems to allows for the formation of dry grasslands of *Festuco-Brometea*. Therefore, in this case the establishment of UE habitat is likely not possible neither by the application of disturbances because of the unsuitability of sites.

G3 cluster group is composed by the relevés corresponding to the highest rockiness, the lowest AWC and the highest EIVs temperature (Table 2 and 2.1). Actually, this plant community develops on the highest hilly slopes with relatively steep morphologies. It has the highest H and S values (Table 2.2) probably because of its fine-scale patchiness.

3 Indicator species and Social Behaviour Types

Although simple measurements of species richness and composition for understanding the ecology of plant communities can achieve misleading conclusions (CAMPETELLA et al. 2004) species grouping by Social Behaviour Types (hereafter SBT) can be a useful tool as well (KIRBY and THOMAS 2000, MOOLA and VASSEUR 2004, GONDARD et al. 2006). SBTs derive from species behaviour and ecological attributes at a given observation level (BORHIDI 1995) and their assessment provides information on the mechanisms underlying species assemblage (KOLASA and ROLLO 1991, ALARD and POUDEVIGNE 2000). Since the higher levels of the phytosociological classification (classes and order) group species that share wide ecological needs and dynamic features, they can be used as useful indicators of SBTs (CATORCI et al. 2011a). In this way, it is possible to couple the phytosociological placement with the SBT assessment of the plant community, thus gaining a deeper understanding of its conservation status. In addition, changes in species composition should be regarded as an outcome of ecological assembly processes, not a causal driver of ecosystem function (MAYFIELD et al. 2010).

To describe not only the species composition but to recognize which species contribute mostly to the composition of the community is useful to highlight the indicator species on the base of their occurrence and abundance. On the basis of fidelity (species that occur only in one group of samples) and exclusivity (species exclusively faithful to group of samples) these species can help to reveal species preferences, determine environmental change and predict vegetation types and impacts of within an area (DE CACERES 2010; DUFRENE and LEGENDRE 1997).

3.1 Materials and methods

To assess the dynamic and conservation state of the plant communities, we grouped species in social behaviour types (SBTs). Species SBTs were assessed considering their regional synecology, following the most accepted phytosociological placement of each species (BIONDI et al 2014, BIONDI & PESARESI 2004, UBALDI 2013). We considered six different SBTs, which are shown in detail by Table 3:

SBT1, species of perennial semi-natural grassland often grazed (species belonging to *Festuco-Brometea*, and *Calluno-Ulicetea* classes);

SBT2, species of xeric, grasslands characterized by Therophytes (*Tuberarietea guttatae* and *Sedo-Scleranthetea* classes);

SBT3, species of meadows (belonghing to *Molinio-Arrhenatheretea* class) usually linked to soil nitrogen enrichment and annual cutting;

SBT4, species of edge habitats (belonging to Trifolio-Geranietea class);

SBT5, characterizing forest recolonization (belonging to *Rhamno-Prunetea* and *Querco-Fagetea* classes);

SBT6, ruderal species (belonging to *Artemisietea vulgaris, Stellarietea mediae* and *Agropyretea intermedii repentis* classes).

SBTs were assessed following the most accepted phytosociological placement of each species considering their regional synecology (PIGNATTI 1982) and some local or wider phytosociological studies (UBALDI 2013, BIONDI et al 2014, BIONDI & PESARESI 2004).

Social behaviour Type (SBT)		
	Phytosociological syntaxa	Abbreviation
SBT1	<i>Festuco-Brometea</i> BrBl. et Tx. 1943	FB
	<i>Calluno-Ulicetea</i> BrBl. & Tüxen ex Klika & Hadac 1944	CU
SBT2	Tuberarietea guttatae	Tg
5612	BrBlRoussine & Nègre 1952	
	Sedo-Scleranthetea BrBl. 1955	SS
SBT3	Molinio-Arrhentheretea Tx. 1937	Mo-Arrh
SBT4	<i>Trifolio-Geranietea sanguinei</i> Müller 1962	TG
SBT5	<i>Querco-Fagetea</i> BrBl. & Vlieger in Vlieger 1937	QF
	<i>Rhamno-Prunetea</i> Rivas Goday & Borja ex Tüxen 1962	RP
	Artemisietea-vulgaris Lohmeyer. Preising & Tüxen ex von Rochow 1951	Av
SBT6	<i>Stellarietea-mediae</i> Tüxen. Lohmeyer & Preising ex von Rochow 1951	Sm
	<i>Agropyretea intermedii repentis</i> Oberd. et al. 1967 Müller et Görs 1969	Ag

Table 3: List of Social Behaviour Type (SBTs) categories with the respective phytosociological classes and abbreviations.

3.2 Data analysis

In order to understand how abiotic factors influence species composition of plant communities, we performed the Indicator Species Analysis (ISA) to highlight the indicator species set (i.e. the species pool showing a preferential distribution among clusters) of each group. ISA is a non-parametric method for identifying those items that show significantly preferential distribution (in terms of frequency and abundance). An Indicator Value (IV) is calculated by multiplying the relative abundance of each item in a particular group and the relative frequency of the item occurrence in the sample of that group (DUFRÊNE and LEGENDRE 1997). The number of randomized IVs higher than the observed ones is used to calculate the probability value (MCCUNE and GRACE 2002). The statistical significance (P< 0.05) of the observed maximum IVs was tested using permutation tests with 4,999 iterations. Only Indicator values higher than 30 were considered of interest.

Furthermore we performed canonical Redundancy analysis (RDA) of the indicator species matrix to compute the percentage of variation in the species abundance explained by the considered environmental factors (see Chapter 2). The indicator species matrix was Hellinger-transformed to avoid considering double absence as a resemblance between sites (LEGENDRE & GALLAGHER 2001).

To identify the indicator SBTs and trait states (aggregated cover values of species sharing the same SBT / trait state) for each group identified by MRT, we executed ISA on this matrix using membership to MRT groups as grouping variable. Finally, to better assess the status of grassland communities, we calculated the relative abundance of each SBT in every considered plant community (cover percent value of the considered SBT out of the total cover value of all the recorded SBTs in the plant community). To perform ISA we used labdsv version 1.6-1 (indval function) R-package.

3.3 Results

Table 3.1 (see Appendix) shows the indicator species identified by ISA for ecological groups highlighted by MRT analysis, as well as their mean abundances. The first and second group of the 3-leaves MRT included ten indicator species; instead, the third was characterized by sixteen indicator species. From a structural viewpoint, *Bromus erectus* dominated both G1 and G3 groups, showing respectively a high cover value of *Brachypodium rupestre* and *Sulla coronaria* as well. Instead, *Avena sterilis* and *Triticum ovatum* dominated the G2 group. ISA of 3-leaved MRT identified several coenological differences (Table 3). Considering the indicator species with the highest mean coverage values (see Appendix Table 3.1) we renamed the ecological groups as follow:

G1-Bromus erectus and Brachypodium rupestre communityG2-Avena sterilis and Triticum ovatum communityG3-Sulla coronaria and Hypochaeris achyrophorus community

RDA executed on indicator species and environmental factors explained 49% of total variance (adjusted $R^2 = 0,16\%$). Constrained variance explained by environmental variables was 13%. The variables showing the strongest correlation with the first two axes were rockiness and altitude that coincides with those discriminating for the partitions of the 3-leaved MRT (see Chapter 2). RDA biplot scores are reported in Table 3.2. The groups highlight by MRT analysis were recognized also in RDA scatterplot (Figure 3)

SBTs of the G1group were: SBT1: species characteristic of perennial pasture, SBT3 species of meadows, SBT4 species of fringe habitat, and SBT5: successional species, SBTs of G2 community were: SBT1, SBT2 species of xeric grassland, and SBT6, G3 group showed only SBT6 ruderal species (Table 3.3)

The abundance (%) of each SBT with respect to the total cover value of the three considered communities is shown in Figure 3.1.

planatory variables are reported for the first three axes.							
	Constraining variables	RDA1	RDA2	RDA3			
	Rockiness (%)	0.895	-0.010	-0.027	-		
	Litter cover	0.492	0.343	-0.144			

-0.043

-0.428

0.800

-0.349

0.790

-0.186

-0.224

-0.327

0.386

0.658

0.442

-0.290

Table 3.2: Biplot scores (scaling 2) for the constraining variables resulting from a Redundancy analysis run on the "species x relevés" and "relevès x environmental variables" matrices. Scores for explanatory variables are reported for the first three axes.

*AWC: Available Water Content

(m

(%)

(°)

(°)

Altitude

*AWC (mm)

a.s.l.)

Slope aspect

Slope angle



Figure 3: Scatterplot of the Redundancy analysis (scaling 2) executed on indicator species of the 3leaved Multivariate regression tree and environmental variables. circles – Group G1; triangles– Group G2; crosses – Group G3. Species codes: *Avena sterilis* (Ave_ste), *Brachypodium rupestre* (Bra_rup), *Broumus erectus* (B_erec), *Hypochaeris achyrophorus* (Hyp_ach), *Linum bienne* (Lin_bien), *Scorpiurus subvillosus* (Sco_sub), *Sulla coronaria* (Sul_cor), *Trifolium campestre* (Tri_cam).

Table 3.3: Indicator social behaviour types (SBTs) of the relevés groups (G1, G2, G3) highlighted by Indicator species analysis for the 3-leaved Multivariate regression tree. SBTs categories are indicated in Table 3

Social behaviour types	Max group [*]	Indicator value ^{**}	P***
SBT1	G1	0.546	0.000
	G2	0.471	0.002
SBT2	G2	0.540	0.003
SBT3	G1	0.500	0.006
SBT4	G1	0.390	0.012
SBT5	G1	0.342	0.004
SBT6	G2	0.729	0.000
	G3	0.497	0.015

*Only significant (P < 0.05) indicator values higher than 30 are shown; **Max group: group with maximum indicator value; *** P: proportion of randomized trials with an indicator value equal to or exceeding the observed indicator value.



Figure 3.1: % abundance of each SBTs in each ecological groups (G1, G2; G3) highlighted by the Multivariate regression tree analysis. SBTs categories are indicated in Table 3.

3.5 Discussion

The three communities highlighted by MRT show a different contribution of SBTs to the species pool (Figure 3.1).

-Bromus erectus and Brachypodium rupestre community

In this community we observed a species composition clearly dominated by entities of dry grassland or meadows (i.e. SBT1, SBT2 and SBT3) as in the semi-natural dry grassland or meadows which are known for the Italian peninsula. In fact, in these communities the average cover value of *Festuco-Brometea* and/or *Molinio-Arrhenatheretea* species generally exceed the 70% (see BIONDI 2012) with a consistent presence of species belonging to *Thero-Brachypodietea* class in the most xeric hilly grassland (BIONDI et. al 2014). In the other groups G3 and G2, species of semi natural grasslands had a relatively low cover value (<50%). It means that the floristic standard needed to ascribe the considered herbaceous plant communities to one habitat of the 92/43/EEC Directive (i.e. 6210) are not still completely achieved, especially in dry pastures (G3)., as indicated by the SBTs assessment (Figure 3.1). Particularly, the more water stressed sites (G3) seem to have large potentiality to host dry grasslands belonging to *Festuco-Brometea* class, while the more productive ones (G1) shown a good suitability for dry meadows of the *Molineo-Arrhenathereta* class.

In accordance with CATORCI and GATTI (2010) these condition allows for the spread of species of SBT1 and SBT3 (Table 3.3) indicating the likeness of these plant communities with the sub-mediterranean hay meadows (see BIONDI et al.2012). However, the absence of disturbance and/or the lack of hay removal after mowing, allows a relatively high value of litter cover (Table 2). Litter can reduce seed germination, establishment of individuals (SILVERTOWN 1980, ERIKSSON 1995), and growth of established plants (FACELLI and PICKETT 1991), thus influencing the processes of species occurrences within plant communities (CATORCI et al. 2011). Likewise, the lack of disturbance enhances the spread of Brachypodium rupestre (Table 3.1). This species is a dominant tall grass, whose competitive success is related to its high tiller density and branching frequency (POTTIER & EVETTE 2010), as well as to its capacity for clonal growth and clonal integration strategy (de KROON & BOBBINK 1997). These features allow a rapid spread especially in high productive conditions (GRIME 2001). The spread of Brachypodium have strong impact on species composition of plant community (CATORCI et al. 2011c, KOSIC et al. 2012) and give rise to the spread of the spread of fringe and successional species (CATORCI et al. 2011a). Actually, SBT4 and SBT5 are indicators of the G1 group (Table 3.3). because it is also undergoing dynamic processes, as indicated by presence among the indicator species of successional species such as Fraxinus ornus and Rubus ulmifolius (Table 3), potentially leading to the formation of woody communities and to the loss of the open habitats.

- Avena sterilis and Triticum ovatum community

This community with intermediate species composition between those of G1 and G3 groups (Figure 3.1), does not seem to have the potentiality to evolve into any habitat of the 92/43/EEC Directive. This fact complies with the presence of SBT2 and SBT6 (ruderal species with wide cover values) and several indicator species of cropland and abandoned field such as (e.g. *Phalaris canariensis, Avena sterilis, Gladiolus italicus, Crepis pulchra, Calamintha nepeta, Potentilla recta*). It indicates the incomplete evolution of the considered communities towards the kind of semi-natural grasslands indicated in the UE Directive. In particular, this herbaceous community seems to have a potential affinity for the *6220 habitat [Pseudo-steppe with grasses and annuals (*Thero-Brachypodietea*)] because it hosts several species of the *Tuberarietea guttatae* class, even if the total cover vale of SBT2 is not very high.

- Sulla coronaria and Hypochaeris achyrophorus community

In this community the xeric group of indicator species as Filago pyramidata, Picris hieracioides, Hypocheris achyriphorus, Thymus longicaulis, Linum bienne, Potentilla hirta (see Appendix Table 3.1) suggest the presence of patches with shallows soils, since it is particularly linked to strong water shortage. Instead, the indicator species Sulla coronaria, Sonchus asper, Sonchus oleracues, Geranium dissectum Crepis vesicaria, Daucus carota besides SBT6 with a very high cover value (Figure 3.1 and Appendix Table 3.1), highlight the past use of these areas as croplands (LAVOREL et al. 1999) and are likely related to the more productive micro-sites. The highest value of litter (see Table 1) is due to the absence of disturbance (WILLIAMS & ASHTON 1987, FACELLI & PICKETT 1991, PREVOSTO et al. 2011) and is related to the permanence of ruderal and early recolonization species, typical of abandoned old fields (SBT6 – indicator trait), since these plants are usually neglected by herbivores or mowing (GRIME 2001). Instead, the low cover value (see Appendix Table 3.1) of dominant tall grasses as Brachypodium rupestre, may be explained with reference to the to the harsh condition of sites (VITASOVICH et al. 2011). This fact confirms the statements that dry condition act as strong abiotic filter (KLIMES et al. 2013) driving to species occurrence and functional convergence irrespective of disturbance regimes.
4 Life forms and morphological features

The potential of plant species to establish or persist under any given set of environmental conditions is determined by their functional traits (MCINTYRE et al. 1999, DÍAZ and CABIDO 2001). Thus, we chose to assess the conservation status of the herbaceous community of the abandoned fields by using morphological features related to vertical and horizontal space occupation as well and life form. These features can explain morphologies and regeneration strategy adopted by plants and are essential to identify general rules freed from particular species identities and based on their biological characteristics (LAVOREL et al.1998). The occupancy in the space reflect natural correlation patterns among attributes and is useful to compare species in the acquisition of resources and as well their plasticity and performances (CAPLAN et al. 2013). Plants life forms, since correlates with others traits, appears to have a largest effect to explain species functionally.

4.1 Material and Methods

In order to explore plant community functional composition, we considered the following traits states: Raunkiaer life forms (chamaephytes, geophytes, hemicryptophytes, therophytes) and type of horizontal space occupation (caespitose, reptant, pleiocorm, rosulate, and prostrate). We also considered the LIIRA and ZOBEL (2000) guilds, as these traits give information about vertical space occupation based on the width of the leaves and on their position along the stem. They are sedges (narrow leaves, leafless stem, most of the leaves are basal), grasses (narrow leaves, leafly stem), rosulate forbs (wide leaves, leafless stem, leaf rosette formed on ground level or higher, leaves mainly horizontally orientated), erosulate upright forbs (wide leaves, leafly stem).We gathered data on traits from the BiolFlor (KLOTZ et al. 2002) and Ecoflora (FITTER et al. 1994) databeses and PIGNATTI(1982)

4.2 Data analysis

As for SBTs analysis (Chapter 3) we transformed morphological traits and life forms states binary data (presence/absence) into quantitative data (i.e. aggregated cover values of each trait state). The "relevés x species cover" matrix was multiplied by the "species x trait states" matrix to provide a "relevés x trait states cover" matrix (PAKEMAN et al. 2009). To identify the indicator trait states for each group identified by MRT, we executed ISA on this matrix using membership to MRT groups as grouping variable (previous Chapter 2 and 3).

4.3 Results

The main morphology of the first community(G1) was caespitose species as horizontal space occupation together with phanerophytes and hemicryptophytes life forms. The second community (G2) showed sedges, grass and prostrates specie as morphologic traits joined to therophytes life forms. Instead geophytes and rosulate life forms belonged to the group G3 (Table 4).

Table 4: Life forms and morphologic features of the relevés groups (G1, G2, G3) highlighted by Indicator species analysis for the 3-leaved Multivariate regression tree.

	Trait state	Max group	Indicator value [*]	Р
Life form	phanerophytes	G1	0.4700	0.005
	hemicryptophytes	G1	0.449	0.000
	therophytes	G2	0.605	0.000
	geophytes	G3	0.473	0.027
Horizontal				
space occupation	caespitose	G1	0.569	0.000
	prostrate	G2	0.472	0.019
	rosulate	G3	0.766	0.000
Vertical				
space occupation	sedge	G2	0.438	0.001
	grass	G2	0.780	0.000
	rosulate	G3	0.642	0.000

^{*}Only significant (P < 0.05) indicator values higher than 30 are shown (Max group: group with maximum indicator value; P: proportion of randomized trials with an indicator value equal to or exceeding the observed indicator value.

4.4 Discussion

In accordance with the results of SBTs analysis and ISA (see Chapter 3) in the first community (G1) the presence of phanerophytes life form and caespitose morphology is related to woody species (e.g. *Fraxiunus ornus, Rubus ulmifolius*) that highlight the early forest recolonization processes of this vegetation while ceaspitose morphology is linked to *Bromus erectus* (occurring in about 96% of plots with mean cover of 40.4%) and *Brachypodium rupestre* (occurring in 77.8% of plots with mean cover of about 23%).

In the G2 community the spread of prostrate plants and sedges (Table 4) is likely due to these environmental conditions as well. In fact, prostrate habitus is the typical growth form of plants growing in water-limited habitats (SKARPE 2001) while sedge growth form increases the probability of locating nutrient-rich pockets and the ability to escape competition for nutrients and water from more aggressive plant species. Moreover, sedges consistently invest great biomass resources in root production and foraging rhizomes to overcome problems of nutrients and water acquisition in poor habitats; they also overcome the issue of the spatial patchiness of resources by adopting clonal integration strategies (MUTHUKUMAR et al. 2004). Furthermore, the presence of the therophytes may be likely explained by the ecological stress of these undisturbed sites.

In the third community(G3) rosulate horizontal morphology of the community, seems not only due to the shallow soil condition (see Chapter 3) but also to the abundant litter coverage caused by the abandonment. Species occupancy in horizontal (as rosettes) occurs in condition with high competition for water soil (see Chapter 2 Table 1; de BELLO et al. 2012) and are more frequent in sites with undisturbed vegetation and rich litter (LAVOREL et al. 1999). Therefore this community appears have a past like croplands and to be in a recolonization stage (see Chapter 3). The spread of geophytes as orchids (i.e. *Anacamptis pyramidalis, Orchis morio, Orchis coriophora*), that are typical of semi-natural dry grasslands belonging to *Festuco-Brometea* class and *Festuco-Brometalia* order (CATORCI et al. 2011, CALACIURA & SPINELLI 2008, BIONDI et al. 2014), suggests the existence of ecological conditions potentially leading to a *Bromus erectus*-dominated community, which is likely referable to the habitat 6210.

5 Functional diversity and vegetation processes

In this chapter the analysis is focused on G1 and G3 groups because the G2 group doesn't reflect properly the species composition of semi-natural grasslands (habitat 6210). Such group is characterized by the conspicuous contribution of species belonging to *Tuberarietea guttatae* and *Sedo-Scleranthetea* classes (see Chapter 3 for details) and the dry environmental condition, at lowest altitudes and on markedly south facing slopes (see Chapter 2).

Only grasslands of the G1 and G3 groups seem to have the potential to develop into seminatural grasslands belonging to the *Festuco-Brometea* class (*6210 habitat). Species belonging to the *Tuberarietea guttatae* class are frequent into the G3 group which shows an ecological proximity to the habitat *6220, which contains pseudo-steppe with grasses and annuals of the *Thero-Brachypodietea*. The G1 and G3 groups are linked to different soil conditions, with respect to pH and AWC (see Chapter 2) that determine the intensity and duration of water scarcity (water deficit) that represent the limiting factor for plants' growing.

5.1 Introduction

Abandoned fields are interesting not only for their conservation status and future restoration practices but also for the evaluation of the spontaneous processes of succession to formulate new and clear theories (ALBERT et al. 2014) on the basis of data from different geographical areas and specific sites (PRACH et al. 2001b, PRACH 2003). We do not know a lot about dynamic trajectories in semi-arid condition and ecologists have not still postulated a unifying theory concerning the changes in species composition after abandonment (BONET 2004, CRAMER et al. 2008). Spontaneus succession from old fields is not always possible, in strong stress condition as well in habitat very productive seems to fail, while seems to realise in intermediate stress-productivity gradient (PRACH and HOBBS 2008) and can be fast with a temporary period of grasslands before the quickly growing of shrub and forest species (SOJNEKOVA et al. 2015).

The specific composition during a successional stage depends not only from local factors as seed disturbance, soil condition (DOLLE et al. 2008) but from their specific traits as well. Functional traits are relevant as they indicate the main plant life strategies adopted to coexist (GRIME & PIERCE 2012, MCINTYRE et al. 1995, CHAPIN et al. 1993,

LAVOREL et al. 2007). They can help to study the vegetation processes (KAMEN & POSCHLOD 2004) and their relationships to the environmental conditions.

Although different traits reveal different functional patterns, convergence or divergence in functional traits can explain the presence of environmental filtering and the acting competition in the community (MARTEINSDOTTIR and ERIKSSON 2014). Their utilization allows to compare different patterns of vegetation and to predict dynamic processes (NOBLE et al. 1996, ROBERTS 1996; CAMPBELL et al. 1999, KLEYER 1999, DÍAZ et al. 2001).

Although there is not an univocal definition of functional diversity, the distribution of functional traits can be considered as a measure of functional diversity (TILMAN 2001, DIAZ et al. 2007). To quantify functional diversity means to evaluate the dispersion of the functional units within a functional space (VILLEGER et al. 2008). This concept has received a wide attention in community ecology (VILLEGER et al. 2008) because can describe the species assemblage including both the information about species diversity and functional traits. The current measures of functional diversity are based on functional groups, i.e. groups of species with same functions and strategies, but not include the differences between them and their abundance else are designed for work only with one character at time (DÍAZ et al. 2001, MASON et al. 2003). For these reasons we evaluate functional traits distribution and functional diversity using community-weighted mean of the traits (CWM) and Rao's quadratic entropy (Rao's Q h) that represent good measures to evaluate functional composition and different aspects of diversity because both include the relative abundance of the species (see section 5.3 for details).

5.2 Hypothesis and Objectives

The maintenance of the diversity within a site is guaranteed by traits differences between species (DE BELLO et al. 2012).

Environmental constraints for resources (light, water...) select species with different functional traits. As well known in dry condition there's a preference in conservative resources strategies e.g. denser tissues, lower canopy height, etc. (CORNELISSEN et al. 2003, FONSECA et al. 2000).

Considering different conditions in terms of water resources in the soil (water deficit) we expect different communities' assemblage after 40 years of abandonment.

5.3 Functional traits

To demostrate the effect of the functional diversity on the community assembly after long-term abandonment we choose plants traits able to highlight aspects as growth, acquisition and allocating resources, life strategies and reproduction.

Leaves traits as SLA (specific leaf area - the ratio of leaf area to dry weight $(mm^2 \cdot mg^{-1})$ and LDMC (expressed here as a percent- age, i.e.) and LDMC (leaf dry matter content - the ratio of leaf dry mass divided by freshmass $mg^*100 \cdot mg^{-1}$) are two of several intercorrelated traits and indicate how plants use resources (de BELLO et al. 2012, PIPENBAHER et al. 2013).

CSR strategies might help to classify species on the basis of the main driving forces as competition (C), abiotic-stress (S) and disturbance (R) (GRIME 1974, MCINTYRE et al. 1995, LAVOREL et al. 1997, WESTOBY 1998); plant height indicates competition for light and means investiment in stem and support structure rather in leaves (WESTOBY et al. 2002); vegetative propagation gives informations about the clonal reproduction and how plants exploit new locations; flowering time might reveal the pattern of coexistence between species in the temporary niche partitioning (CATORCI et al. 2012), seed weight is linked to sexual reproduction and dispersal ability (GRIME 2001).

5.4 CWM and Functional diversity (Rao's Q index)

CWM and Rao's Q are considered good candidates to summarize functional aspects between communities. CWM represents the average for each traits within a community and Rao's coefficient indicate rather the distribution of the functional traits (RICOTTA & MORETTI 2011). If we considered species inside a functional space these indeces describe different aspects: CWM highlights the variantion in mean of functional composition rather Rao's Q evaluate the functional diversity calculating the average pairwise differences between two species (BOTTA DUKAT 2005).

5.5 Functional diversity components

For a more accurate evaluation to split functional diversity between three primary components as Functional Richness (FRic), Functional Evenness (FEve) Functional Divergence (FDiv) provide more details on the linkage between biodiversity and ecosystem functioning (VILLÉGER et al. 2008). These measures are complementary characteristics of the distribution of species in a niche space (MOUCHET et al. 2010, MASON et al. 2005); FRic represents the amount of the niche occupied by the species assemblage, FEve is the regularity with which the resources are utilized by the species into a functional niche and FDiv indicates the space between the species and the center of the functional space, therefore the divergence of the distribution of the species traits (MASON et al. 2005, MOUCHET et al. 2010, PAKEMAN 2011). Several theorethical bases on these measures are available but few testing with field data and evaluations on how they respond to environmental condition occur until now (PAKEMAN 2011). This supports the demand to test their capacity to explain interactions between species and ecosystem functions (Mouchet et al 2010).

5.6 Materials and Methods

5.6.1 Summer water deficit

To calculate the deficit of water in the soil during the summer period (from May to August) we used the software developed in Microsoft Office Excel 2000 by ARMIRAGLIO et al. (2003). We used the same soil parameters and climatic data for the calculation of AWC (see section 2.2) because the deficit of water is represented by the difference between the potential evaporanspiration and the real evapotranspiration (ARMIRAGLIO et al. 2003).

5.6.2 Traits selected

We considered seven key traits following several literature sources (HODGSON et al. 1999, KAHMEN et al. 2002, CORNELISSEN et al. 2003). Traits were chosen from our database following the standardized protocols of Cornelissen (2003) and from the existing databases as BiolFlor (KLOTZ et al. 2002), LEDA (KLEYER at al. 2008), the Royal Botanic Garden at Kew's Seed Information (www.kew.org/data/sid/sidsearch.html) and e-florasys (PLANTUREUX and AMIAUD, 2010, http://eflorasys.inpl-nancy.fr/). We calculated CSR strategy following the practical tools of HUNT et al. 2004. All traits selected are shown in Table 5.

Table 5: Seven	functional	traits ar	d 19	categories	recorded	for	135	vascular	species.
Scales of measure	rement were	e binary	and c	ontinuous.					

Traits	Abbreviation and Description	Data source	Type of Data
Type of vegetative propagation	vp-n: absence (only sexual reproduction) vp-uo: root tuber, stem tuber, root splitter, bulb vp-r: runner, runner like rhizome (<i>epigeogenous stem</i>) vp-rh: rhizome (<i>hypogeogenous and</i> <i>epigeogenous stem</i>), root shoot, rhizome- like pleiocorm	BiolFLor (Klotz et al., 2002)	binary
Plant height (cm)	h<20 cm h-21-40 cm h-41-60 cm h-61-80 cm	Own measurements and Pignatti (1982)	
Flowering length	lw: later winter (February-March) f-es: early spring (March-April) f-mls: spring flowering (May-June) f-esu: early summer (June-July) f-mlsu: summer flowering (July-August)	Pignatti (1982)	
Seed weight (mg)	SW	Kew Seed Bank database	continuous
Specific leaf area	SLA (mm ² /mg)	LEDA database (Kleyer et al., 2008); own measurements	
Leaf dry matter content	LDMC (mg/g)	LEDA database (Kleyer et al., 2008); and own measurements	
Grime's strategy	C: competitive S: stress tolerant R: ruderals	Hodgson et al. (1999)	

5.6.3 CWM and Functional diversity (Rao's Q index)

To evaluate functional composition of the vegetation we considered two approaches: first we calculate the community-weighted mean trait values for each relevé (CWM) as follows:

$$CWM_{jk} = \sum_{i=1}^{S} p_{ik} * x_{ij}$$

where CWM_{jk} is the community-weighted mean value of trait j at site k, pik is the relative abundance of species i (i = 1, 2,..., S) at site k and *xij* is the value of trait *j* for species *i*. (RICOTTA & MORETTI 2011). The output of this calculation was a matrix 80 relevès x 19 traits (CWM matrix). Second we considered functional diversity (FD) using Rao's (1982) quadratic entropy as follows:

$$FD_Q = \sum_{i=1}^{S} \sum_{j=i+1}^{S} d_{ij} p_i p_i$$

where FD_Q expresses the average difference between two randomly selected individuals with replacements (BOTTA-DUKÁT 2005), S is the number of the species, *dij* is the difference between the *i*-th and *j*-th species (*dij* = *dji* and *dii* = 0), p_i and p_j are the proportion of the species *i*- th and *j*- th in the community. The output of this calculation was a new matrix of 80 relevés x 19 FD values (FD matrix).

5.6.4 Functional diversity components

We considered the three components of FD for each releves: Functional Richness (FRic), Functional Evenness (FEve) Functional Divergence (FDiv). If we consider a multi-traits approach and a convex hull volume as a measure of the functional space occupied by a given community (CORNWELL et al., 2006) FRic and FDiv both rely on finding the minimum convex hull that includes all species and FEve quantifies the regularity with which the functional space is filled by species, weighted by their abundance (VILLÉGER et al. 2008).

5.7 Data analysis

5.7.1 Data treatment

To provide a visual representation of differences in summer water deficit we applied Canonical correspondence analysis (CCA, TER BRAAK 1987). Ordination was applied to the 135 species x 80 relevés and 80 relevés x water deficit (mean value for each month from May to August) matrix. The significance of all canonical axes were tested by the Monte Carlo permutation test which evaluate the independency between species x releves matrix and releves x water deficit matrix under null model assumption. Ordinations method (CCA) and graphic were carried out using the Canoco for Windows version 5.0 and CanoDraw for Windows version 4.5 programs (TER BRAAK and SMILAUER 2002).

5.7.2 CWM and Functional diversity (Rao's Q index)

To test differences of the plant functional traits between two groups defined with CCA analysis was performed RLQ analysis (LEGENDRE et al. 1997). We used simultaneously three matrices: 135 species x 80 relevés, CWM matrix (80 relevés x 19 traits), 80 relevés x 2 groups (as dummy variables). RLQ measures the link between environmental variables and species traits: for continuous traits tests whether the F-statistic for trait differences between communities is greater than expected under the null models used, instead for binary traits uses Pearson's Chi square test.

To investigate the differences between the groups on the basis of functional diversity Rao's Q index we analyzed FD matrix (80 relevés x FD values traits) with one-way ANOVA. Before ANOVA test we checked homogeneity of variances with Levene's test. When homogeneity was not respected we performed a logarithmic transformation, otherwise we advanced assuming no equal variances. Statistical analysis were calculated in R software (version 3.2.0 - R Core Team 2015 - http://www.R-project.org) using Rcmdr and stats (ANOVA), FD (Rao's Q), ade4 (RLQ) packages.

5.7.3 Functional diversity components

Analysis one-way ANOVA was used for three complementary components of functional diversity (FRic, FEve and FDiv). Before ANOVA test we checked homogeneity of variances with Levene's test. When homogeneity was not respected we performed a logarithmic transformation, otherwise we advanced assuming no equal variances. Statistical analysis were calculated in R software (version 3.2.0 - R Core Team 2015 - http://www.R-project.org) using Rcmdr and stats (ANOVA), FD (FRic, FEve and FDiv), car (Levene's) packages.

5.8 Results

5.8.1 Comparison in summer water deficit

Across 80 relevés we identified 135 vascular plant species among four study areas. Differences in species composition and water deficit we analysis with CCA ordination (Figure 5). The CCA axis 1 of this analysis showed clear division into two groups. Eigenvalues for the first two CCA axes are 0.28 and 0.41. Monte Carlo permutation test was significant for all axes (p=0,002).



Figure 5: CCA ordination diagram on two matrices: 135 species x 80 relevés and 80 relevés x water deficit. Eigenvalues: axis 1=0.28, axis 2=0.41; p=0,002 of Monte Carlo permutation test. Relevés split in two groups: triangles - G1, circles - G3

5.8.2 CWM and Functional diversity (Rao's Q index)

RLQ analysis showed significant differences of the CWM values between both groups: sexual reproduction (vp-n), species that flowering in early spring (f-es) are higher in the group 2, instead LDMC, vegetative propagation strategies (vp-r, vp-rh) characterize the group G1 (Figure 5.1). A marginally significant value was shown also by ruderal species (p=0,08) that is higher in the group G3.

The comparison between both groups on Functional Diversity values (Table 5.1) highlighted further differences: all values of FD for all traits were higher for the group G3 except for vegetative propagation vp-uo and vp-r (see Table 5 for abbreviation) that were higher in group G1.



Figure 5.1: Boxplots of CWM traits values for differences between groups. Only significant traits are shown. P values from RLQ analysis on three matrix: 135 species x 80 relevés, CWM matrix (80 relevés x 19 traits), 80 relevés x 2 groups. 1 = G1, 2 = G3. All the abbreviations of plant traits categories are in Table 5.

Table 5.1: One-way ANOVA of FD matrix (80 relevés x 19 FD values). Mean and standard deviation (sd) are indicated for each groups. All abbreviation of plant traits categories are in Table 5. Only significant traits are shown. significance code0= '***'; 0,001 = '*'; 0,01 = '*'; 0,05 = '

Traits	Traits	Groups	mean	sd	F value	р
	Abbreviation					
Plant		G1	0,268	0,147	26,46	***
height						
	h20	G2	0,567	0,326		
		G1	1,108	0,267	4,731	*
	h41-60	G2	1,230	0,235		
	h61-80	G1	0,433	0,648	16,15	***
		G2	1,283	1,136		
Flowering	f-esu	G1	0,632	0,239	18,91	***
time		G2	0,831	0,169		
		G1	0,023	0,094	33,9	***
	f-lw	G2	0,498	0,488		
		G1	0,405	0,265	110,2	***
	f-mls	G2	0,954	0,202		
	f-mlsu	G1	0,819	0,160	10,49	**
		G2	0,919	0,116		
Vegetative	vp-n	G1	0,502	0,254	20,61	***
propagation		G2	0,763	0,257		
	vp-r	G1	1,301	0,608	88,2	***
	1	G2	0,265	0,365	,	
	vp uo	G1	0,685	0,593	30,58	***
	1_	G2	0,164	0,162	,	
LDMC		G1	0,602	0,210	15,09	***
		G2	0,833	0,303	, i i i i i i i i i i i i i i i i i i i	
SLA		G1	0,354	0,207	34,85	***
<u><u>J</u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>		G2	0,832	0,453	, i i i i i i i i i i i i i i i i i i i	
seed weight	SW	G1	0,845	1,484	13,74	***
0		G2	2,097	1,526		
Grime's	С	G1	0,392	0,148	16,07	***
strategy		G2				
0.			0,559	0,214		
	S	G1	0,569	0,256	4,274	*
		G2	0,698	0,297		

5.8.3 Functional diversity components

The three components of FD for continuous and binary traits showed differences as well: FDiv, FEve for binary traits values were higher in group 1 instead FRic for continuous traits values was higher in the group 2. Marginally significance was showed for FRic for binary traits values (Figure 5.2).



Figure 5.2 Boxplots of FD indexes differences between groups:1=G1, 2=G3. Only significant values are shown: P values from one-way ANOVA. FDiv_bin = Functional Divergence for binary traits, FEve_bin = Functional Eveness for binary traits, FRic_bin = Functional Richness for binary traits and FRic_con t= Functional Richness for continuous traits. Explanation of binary and continuous traits is in Table 5.

5.9 Discussion

The functional diversity approach at every level, CWM, functional diversity (Rao's Q index) and functional diversity components (FDiv, FRic, FEve) demonstrated the existence of two group of species with different assemblage after 40 years of abandonment in relation to different conditions of water deficit in the soil, one of the most important factor that control heterogeneity in plants distribution (NOY-MEIR 1973, ZOHARY 1973).

5.9.1 CWM, Functional diversity and Functional diversity components

If we consider the CWM, i.e. the average of trait values weighted by the relative abundance of each species, (LEPS et al.2006, LAVOREL et al. 2008, RICOTTA and MORETTI 2010, 2011) the species of the first group adopt mostly vegetative reproduction with rhizome or running structures (vp-r, vp-rh) and shown higher LDMC. The spread of runner structures occurs when the availability of resources is high (GRIME 2001) and enables plants to explore unexploited niches (TISSUE & NOBEL 1988, FRIEDMAN & ALPERT 1991) above all when there is an higher exploitation of the resources in the soil (CATORCI et al. 2012). In relation to the water deficit is possible that vegetative propagation in this community is linked to the strategies to uptake water in the soil that is more available (less water deficit) and maybe more patchy distributed. Leaf dry matter content (LDMC) is related to leaves density and their potential growing (CORNELISSEN et al. 2003). Higher values of this trait indicate a slow retention strategy for nutrient elements (PIPENBAHER et al., 2013, POORTER & GARNIER 1999). LDMC is negatively correlated with the specific leaf area (SLA) that in our case shown the higher value of FD in the group G3. If we compare this two results (LDMC and SLA between groups) in relation to the resources exploitation seems that species of the first group have more conservative strategy than the second group (G3), where species show instead a fast-growing strategy (high SLA and low LDMC considering CWM and FD both). In the second group (G3) species show furthermore higher values of FD for stress-competitor C (competitive) strategy and S (stress-tolerant) strategy. Plants' height, SLA C and S strategies are linked traits. The different significant range of plants height highlights the competition towards with this resource. Taller plants with higher SLA win in the interception of light and show competition effect due to the greater

interception (ADAMS et al., 2007;VIOLLE et al.,). SLA indeed represents a measure of the interception of the light per leaf dry mass unit (REICH et al., 1997) thus higher SLA means to be more able to capture light. But the intense exposure to the light, due to the topographic features(south facing) and lower availability of water in the soil, above all during spring and summer, represent stress factors for growing and justify the presence of higher values of S strategy as well. In contrast, smaller plants tolerate the shading of the plants neighboring and are able to grow when perceive a change in light (REICH et al., 1992) or because they have adapted structure and physiology (WERGER et al., 2000).

The competition for light could explain also the higher CWM values for species with early spring flowering (f-es). In light strong condition, early growing (early spring) is ensured by the moving of the reserves from the storage organs allowing a rapid foliage expansion and a canopy preemption resulting in a competitive advantage (GRIME 2001). The most common reproduction strategy of this community is indeed a reproduction by seed as highlighted by higher FD values of seed weight and the absence of vegetative propagation (higher CWM values for vp-n) and there is a continuous growing during the summer season (higher FD values for every category of flowering time traits). On these findings it's possible to state that species move the reserves accumulated in storage organs (such as seed) during the previous growing season and flower in diachronic way resulting in a better differentiation of the temporal niche and ensure themselves resources for growing.

Considering the three components of the FD in the second group (G3), the functional richness (FRic) is higher than in the first group (G1). Following MASON et al. 2005 in terms of resources this means that whole "alpha niche". i.e. all the potential resources available, are used by the species. However the resources are better distributed within the niche space in the first group(G1) as demonstrated by higher functional divergence (FDiv) and functional eveness (FEve). These results are in accordance if we look to the reproduction through hypogeogenous and epigeogenous stem on the ground adopted by the species which allow a more equipartition in the resources uptake respect to sexual reproduction by seed (in the group G3), that conversely ensure more accumulation of resources (ERIKSSON et al. 1997, MOLES and WESTOBY 2004) and guarantees higher establishment of success (MARTEINSDÓTTIR and ERIKSSON 2014).

5.9.2 Functional diversity and succession

Referring also to the structure of the vegetation of the second community (G3) with scattered shrubs and trees, together with our results respect to seed more weight and

higher CWM values for ruderal species (even if with a marginally significance) is possible to understand that this abandoned grasslands occurs an early stage of succession. Previous studies about natural succession from the former arable field, affirm that the first stage of the natural succession starts with perennials ruderals species (DOLLE et al. 2008) and with a seed dispersal strategy to reproduction (ALBERT et al. 2014). In comparison the community of the first group (G1) seems to be in later stage of succession because dominant grasses and forbs seem to replace ruderal perennials species (BROWN and SOUTHWOOD 1987, SCHMIDT 1993) and the dispersal seed strategy is replaced by the clonal ability (ALBERT et al. 2014). This is in line with our findings related to CWM values for LDMC and vegetative propagation in this group. Higher LDMC values are linked with more conservative species (DIAZ et al. 2004; WRIGHT et al. 2004) and more retention strategy (PIPENBAHER et al. 2013) and clonal ability results more important for local persistence and spread (LINDBORG et al. 2012) when there are not constraints for water acquisition (lower water deficit). Thus this first group seems to be in a more stable stage in terms of natural succession.

5.9.3 Functional diversity and disturbance

Despite these grasslands are abandoned by time (30 years) evidences of disturbance in the past are still present. Both indices used on traits (CWM and FD) have shown that functional diversity is greater in the community G3. The influence of disturbance is highlighted by the presence of ruderal species and the lower values of CWM for LDMC. In accordance with literature species with lower values of LDMC and higher proportion of plants with R (ruderal) strategy are associated with high disturbance regime condition. Lower LDMC can be linked to higher SLA values. Leaves with low LDMC values and high SLA are more soft and more suitable to be grazed than those with high LDMC that tend to be quite tough and are assumed to be more resistant to biotic (herbivory, wind) and abiotic (wind, mowing) hazards than leaves with low LDMC (PEREZ-HARGUINDEGUY, 2013). The disturbance regime favors also a species with persistence green leaves that flower in early spring (see CWM for f es) and in later winter (see FD for f lw CWM and FD for f es) besides that in summer. Species therefore try to exploit resources in staggered periods to fulfill the stages of growth, such as flowering. Although in the literature it is argued that the species with C (competitive) strategies are frequent in low disturbance regime in G3 community this strategy occur as well. This trait could be explained as a competitive effect traits related to the depletion of the resources (NAVAS et al., 2009). This community grow on more hash environmental condition caused by the highest rockiness and litter cover, and the lowest AWC values, as

well as by low soil moisture EIVs (see Chapter 2) that determine limiting condition for resources uptake, especially as explained for light and water in section 5.9.1.

5.10 Conclusions

Considering all our results it is possible argue that abandoned grasslands with different ecological constraints as the water availability in the soil reach different functional assemblages after the 30 years of absence of management.

The community (G1) with more availability of water in the soil, and more conservative strategies (LDMC, vegetative propagation) favorable to persistence and to the local spread, with most equal distribution in resources exploitation.

The community (G3) with a more functional heterogeneity and competitive strategies due to the more limiting condition of water and intense light, where all potential resources are exploited, inside an early natural process of succession, where the disturbance of the land use history is still visible.

The functional diversity at different levels (CWM, Rao's Q index and FD components) allows thus to discover deeply the strategies acting in the communities even if more information about limiting factors are necessary to clarify further relations between species and ecosystem functions (MOUCHET et al. 2010).

6 Final conclusions

Different approaches that we used demonstrated that in our study area the abandoned fields included in the Natura 2000 regional network as habitat *6210 (Semi-natural dry grasslands and shrubland facies on calcareous substrates - important orchid sites) with correspond to different plant species composition as a result of different environmental conditions. Through SBT's, EIV's values and ISA we provided information on the mechanisms underlying species assemblage useful to clarify the ecological meaning of the species pool characterizing a certain plant community (CATORCI et al. 2011a). Furthermore morphological traits and functional diversity between the community G1 and G3 helped us to reveal deeply the assemblage species and the acting forces that shape their spatial pattern in relation to different ecological conditions of availability of the resources, soil and topographic features.

6.1 Vegetation features

Only the first community (G1) with the dominance of *Brachypodium rupestre* and *Bromus erectus* seems to reflect the composition of the habitat 6210 (92/ DIRECTIVE). It corresponds to moderately xeric conditions, which that allow the spread of *Festuco Brometea* species (UBALDI 2013, CATORCI et al 2011 c, BIONDI and GALDENZI 2012). However these grasslands, as testified by the presence of species of fringe habitat and succession species as *Fraxinus ornus* and *Rubus ulmifolius*,(see Chapter 3-4) indicate unstable dynamic stages, have small extensions and could undergo a scrub enrichment without an appropriate management that could allow to maintain open areas. This is supported as well by the functional traits analysis: species show conservative strategies for leaves (LDMC, SLA), vegetative propagation with runner structures for reproduce and explore neighboring area (CATORCI et al. 2012) and as occur in the next succession process, the ruderal perennial species are replaced by competitive grasses and forbs (BROWN and SOUTHWOOD 1987, SCHMIDT 1993).

The second community (G2) dominated by *Triticum ovatum* and *Avena sterilis* seems to have the potentiality to develop in *6220 habitat [Pseudo-steppe with grasses and annuals (Thero-Brachypodietea)] because it hosts several species of the *Tuberarietea guttatae* class. However the presence of several species of croplands and abandoned fields (e.g. *Triticum ovatum, Phalaris canariensis, Avena sterilis, Crepis pulchra, and Scorpiurus subvillosus*) indicates the incomplete evolution towards the kind of semi-natural grasslands indicated in the UEDirective.

The third community (G3) dominated by *Hypochaeris achyrophorus* and *Sulla coronaria* seems to have a past life history as croplands as testify by several species (i.e *Sonchus oleraceus, Geranium dissectum, Crepis vesicaria, Daucus carota)* but the spread of geophytes, such as orchids (i.e. *Anacamptis pyramidalis, Orchis morio, Orchis coriophora)*, besides the relatively high cover value of *Festuco-Brometea*, *Tuberarietea guttatae* and *Sedo-Scleranthetea*, suggest the existence of ecological conditions that potentially could lead to xeric *Bromus erectus*-dominated community (which could be referred to the *6210 habitat). However the presence of successional species (6 %-see Chapter 4) are in unstable dynamic stage as it show by the analysis of some functional traits. In this community indeed the preference of the species for sexual reproduction (seeds reproduction) and the presence of the ruderal species are significant factors in the first stage of the natural succession (DOLLE et al. 2008, ALBERT et al. 2014).

Neglecting the *Avena sterilis* and *Triticum ovatum* community (G2) because not corresponding properly to *6210 habitat, the functional diversity analysis between G1 and G3 communities highlights different functional strategy in relation to the content of water in the soil (summer water deficit) one of the limiting factor for plants' growing.

The community (G1) showed more conservative strategies for leaves (LDMC) and vegetative propagation. These features are favorable to the local spread and persistence and allow a most equal distribution in resource exploitation (as indicated by Functional divergence, Functional evenness). The community G3 showed a more functional heterogeneity (Functional richness) and competitive species (competitive and stress tolerant species value) due to the more limiting condition of water and intense light that justify also the higher Specific leaf area (SLA)and plant height values.

6.2. Vegetation dynamics

Considering the interplay between species, environment and the functional responses of the species it possible to argue that the sub-Mediterranean hilly abandoned fields have a good potentiality to develop into different UE habitats, notwithstanding to complete such process the systems must undergone to appropriate disturbance and management regimes.

The absence of disturbance provided a double effect. Following SMITH et al. (2000) it allowed the spread of woody successional species (shrubs and pioneer trees) and leads to forest recovery as well as the permanence of plants with ruderal-like ecological needs. Likewise it allowed the spread of competitive species which reduces the site suitability for non-dominant herbaceous plants (such as small-sized and early flowering species– GRIME, 2001). These are key elements of the grasslands referred to the 6210 priority habitat protected by the 92/43/EEC Directive.

The spread of competitive grasses (i.e. *Brachypodium rupestre*) fosters the presence of elements, such as fringe species, that are not characteristic of semi-natural disturbed grasslands (CATORCI et al. 2011a).

Instead, the dominance of species of old abandoned fields belonging to *Artemisietea* and *Agropyretea* syntaxa which characterize the first stages of natural recolonization processes (JI'ROVA' et al. 2012) indicates that more time from cropland abandonment is needed to reach a significant change in the species composition.

Time after abandonment is considered a key issue in semi-natural grassland recovery but probably tall plants with ruderal-like ecological requirements are also able to persist for longtime in abandoned field without disturbance (GRIME 2001). Nevertheless, although recolonization processes are not sufficient per se to habitat recovery, they drive to semi-naturallike herbaceous communities whose species compositions are strongly linked to the soil water availability and landforms. Particularly, on steep slopes semi-natural dry grassland belonging to the *Festuco-Brometea* class and thus the 6210 habitat can often establish. Otherwise semi-flat areas mainly with north-facing exposures seem offer suitable sites for hay meadows (*Molinio-Arrhenatheretea* class).

6.3. Management suggestions

As regards to management practices sheep or cows should graze on grasslands belonging to the G3 group during the late spring and summer periods to reduce the abundance of dominant tall plants and enhance species with avoidance and tolerance strategies, which are species of the *Festuco-Brometea* class (GRIME 2001). Sheep, in particular, are known as dispersal vectors for most of the species of the *Festuco-Brometea* class (FISCHER et al. 1995), and thus they can facilitate the processes related to species spread in managed grasslands (KALIGARIC et al. 2006). Grasslands of the G1 and G2 groups should be mown once a year in early summer, since late mowing allows the conservation of the maximum species richness (CATORCI et al. 2014a). Moreover fresh phytomass should be removed to avoid litter deposition and summer grazing can be useful to achieve a higher level of representativeness. With reference the two grasslands (G1 and G3) closer to the habitat 6210 the conservation priority is better to reduce shrubs propagation, where established protected or endangered orchids species as *Anacamptys pyramidalis, Orchis coriophora, Ophrys fusca*.

With these findings it will be possible to focus economic resources for monitoring communities closer to 6210 habitat that respect more the standards of conservation set by the 92/43/EEC Directive or evaluate the representativeness of other community (i.e. *Avena sterilis*

and *Triticum ovatum* community) with respect to the aspects of greater conservation interest of other habitat of Natura 2000 Network. In this way the Italian interpretation manual of the 92/43/EEC Directive habitats (BIONDI et al. 2009) could be update and improved.

About the possibility of the restoration of semi-natural grasslands from abandoned fields, more empirical studies are needed to better understand if seeds supply or modification of the soil chemical features are needed. Concerning the vegetation process that act on the species composition an own specific database functional traits is necessary to define better changing in the local communities during the natural succession.

We conclude that these abandoned fields have a good potential to develop into Habitat of EU Directive but without appropriate management plans they will remain of low representativeness.

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Pubblications

Troiani N., Malatesta L., Corazza M., Ferrari C., Catorci A., 2016: Grassland recovery after long-term cropland abandonment. Acta Botanica Croatica, (in press).

Troiani N., Malatesta L., Corazza M., Catorci A., How environmental factors drive the floristic composition and dynamics of semi-natural dry grasslands in the hilly landscape of Tuscan-Emilian Apennines. *International Symposium of Floristic Patterns at different organisation and distribution levels*", 16-18th May, 2014, Babeş-Bolyai University ,Cluj-Napoca Romania, (abstract).

This research aimed revealing the dynamic status of semi-natural dry grasslands and assessing how environmental factors shape their species composition in five sites (Sites of Community Importance - SCI and Special Protection Areas - SPA) of Natura 2000 Network (92/43/EEC Directive). In order to provide data about their conservation status, possible management measures and related impacts on these sites. To achieve these goals, 100 relevés (100m² plots) were carried out and topographic factors (altitude, slope aspect, and slope angle), degree of rockiness, soil physical factors jointed with climatic variables (soil Available Water Content) and proxies to land use (litter abundance) were collected. The Ellenberg's ecological indicator values, and three functional traits (life form, Social Behaviuor Types, vertical/horizontal space occupation) were assessed for all species set. The data were statistically analyzed by Multivariate regression tree analysis, Indicator Species analysis, Redundancy analysis and Detrended Correspondence Analysis.

All the analyses discerned three clusters of communities with different dynamic status: *Festuco-Brometea* meadows with functional traits that highlighted their successional stage (bush encroachment development); post-abandonment, ruderal communities represented by *Stellarietea-mediae* and *Artemisietea vulgaris;* communities with intermediate status between the previous two.

Our results demonstrated that the species assemblage of dry semi-natural grasslands in the Tuscan-Emilian Apennines is mainly driven by environmental features (i.e topographic factors, degree of rockiness, soil physical factors jointed with climatic ones and land use). Some plant traits are closely associated with response of vegetation to land abandonment dynamics, irrespective of the type of habitat. We hypothesized that species richness is negatively affected by the bush encroachment development. On the basis of our findings the knowledge of the coenotic differences due to life-history and environmental features should be considered a powerful tool in management planning, in order to maintain the biodiversity of these communities.

Tardella FM., Troiani N., Malatesta L., Piermarteri K., Postiglione N., Ferrari C., Catorci A., Long-term abandonment of croplands in the sub-Mediterranean climate does not lead per se to the recovery of the semi-natural herb communities deemed worthy of conservation in the EU Habitats Directive. 25th meeting of European Vegetation Survey, 4-6th April 2016, Rome, Italy, (abstract).

Abandoned croplands can be considered a new category of "scattered elements" of mountain landscapes. To gain deeper understanding of the conservation status (sensu EEC Directive 92/43) of abandoned cropland in the northern Apennines, we coupled the concepts of the social behavior type (SBT) and the functional assessment of plant communities. SBTs refer to behaviour and ecological attributes of species at a given observation level and allow the understanding of the plant community conservation status, while the functional approach may help in predicting changes of species composition along disturbance and stress gradients. We found that topographic and soil conditions drive the species assemblage in pastures after crop abandonment, but long-term abandonment does not lead *per se* to the recovery of the semi-natural grassland communities deemed worthy of conservation in the EEC Directive. It was mainly due to the lack of appropriate disturbance regimes that allows the spread of dominant tall herbs, which, in turn, reduces the site suitability for subordinate plants. Moreover, their spread fosters the presence of elements such as ruderals and fringe species. We conclude that, these abandoned croplands have a good potential to develop into Habitat of EU Directive but without appropriate management plans they will remain of low representativeness.

Appendix 1

Table 3.1: Indicator species of the relevés groups highlighted by Indicator Species Analysis for the 3-leaved Multivariate regression tree and their respective indicator values and mean cover percentage, as highlighted by the indicator species analysis.

				Mean
Max	Indicator	Indicator		cover
group*	species	value**	P^{***}	(%)
G1	Brachypodium rupestre	0.66	0.00	22.94
	Achillea collina	0.53	0.00	0.99
	Cota tinctoria	0.49	0.01	1.54
	Bromus erectus	0.44	0.01	40.44
	Vicia sativa	0.39	0.01	0.45
	Lotus corniculatus	0.35	0.01	0.64
	Securigera varia	0.33	0.01	0.31
	Inula salicina	0.32	0.01	0.87
G2	Triticum ovatum	0.77	0.00	14.90
	Avena sterilis	0.64	0.00	32.10
	Scorpiurus subvillosus	0.47	0.00	2.70
	Hippocrepis comosa	0.46	0.00	0.50
	Calamintha nepeta	0.41	0.01	2.20
	Gladiolus italicus	0.38	0.00	0.13
	Potentilla recta	0.38	0.00	0.31
	Phalaris canariensis	0.35	0.00	0.40
	Trifolium campestre	0.35	0.04	1.50
	Crepis pulchra	0.30	0.02	0.32
G3	Linum bienne	0.85	0.00	3.65
	Hypochaeris achyrophorus	0.74	0.00	11.53
	Sonchus asper	0.68	0.00	0.68
	Picris hieracioides	0.67	0.00	0.67
	Allium schoenoprasum	0.65	0.00	0.41
	Sulla coronaria	0.63	0.00	19.02
	Potentilla hirta	0.54	0.00	0.61
	Daucus carota	0.50	0.00	0.88
	Althea hirsuta	0.49	0.00	0.65
	Geranium dissectum	0.44	0.01	1.16
	Bartsia trixago	0.40	0.01	0.35
	Sonchus oleraceus	0.40	0.00	0.46
	Thymus longicaulis	0.39	0.02	0.68
	Ononis masquilleri	0.36	0.01	1.60
	Crepis vesicaria	0.33	0.02	0.31
	Filago pyramidata	0.31	0.03	0.40

*Max group: group with maximum indicator value; **Only significant indicator values (P < 0.05) higher than 30 are shown; ***P proportion of randomized trials with an indicator value equal to or exceeding the observed indicator value